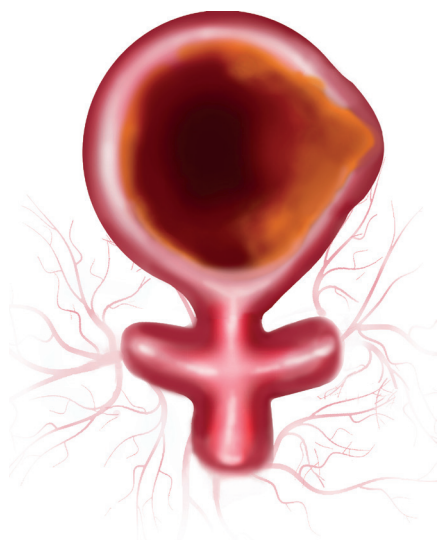


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## **Unfolding Lipid Profile- and Sex-Paradoxes in the Epidemiology of Subarachnoid Haemorrhage**



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HELSINKI UNIVERSITY HOSPITAL  
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# **Unfolding lipid profile- and sex- paradoxes in the epidemiology of subarachnoid haemorrhage**

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# Abstract

## Introduction

In cardiovascular diseases, high total cholesterol (TC) and male sex usually associate with elevated risk. However, studies of aneurysmal subarachnoid haemorrhages (aSAHs), report paradoxically that low TC and female sex elevate aSAH risk. Additionally, no population-based studies on the risk factors behind the most-fatal aSAH: sudden-death aSAHs, exist. This thesis aims to unfold these two paradoxes and aims to characterize the risk factors of sudden-death aSAHs using population-based cohort that includes also autopsy records of all sudden-death individuals.

## Methods

A systematic review and meta-analysis identified the key studies included in the literature review and in hypothesis formation. The Preferred Reporting Items for Systematic reviews and Meta-analyses (PRISMA) check-list guided the reporting, and according to the Cochrane Collaboration guidelines, the review article focused on qualitative analysis because of high study heterogeneity. In cohort studies 65 521 population-based FINRISK participants were followed prospectively from medical registries since 1972 until the end of 2014. Participants enrolled at five-year intervals and provided 543 incident aSAHs, of which 98 were sudden-death aSAHs confirmed by computer tomography, spinal tap, or at autopsy. Baseline measurements at enrolment provided risk factors and potential confounders for the analyses and multiple imputations supplemented the missing values. The Cox regression, adjusted for the main risk factors and confounders, provided hazard ratios and a platform for population-attributable fraction, multiplicative effect modification, and additive effect modification analysis. Competing risks analysis mapped the associations between risk factors and aSAH during a long follow-up.

## Results

The systematic review found many high-risk-of-bias studies describing that low TC elevates aSAH risk, but it detected only two low-risk-of-bias studies, both indicating that high TC elevates aSAH risk. The cohort study, which includes more-detailed lipid profile analyses, supports the review's findings and suggests that the adverse lipid-profile plays a considerable role in aSAH development especially in men. Smoking had a linear dose-dependent association with aSAH in both sexes, and ex-smokers had reduced aSAH risk when compared to smokers. However, although smoking elevated aSAH risk more in women, female never-smokers were not at elevated risk, indicating that in women effect modification between sex and smoking explained the elevated risk. Along with high systolic blood pressure, smoking also elevates sudden-death aSAH risk more than it elevates risk for hospitalized aSAH.

## Conclusions

Methodological limitations in earlier studies explain at least in part the paradoxical, risk-increasing association between low TC and aSAH. An adverse lipid profile is an important aSAH risk factor and accounts for a major fraction of all aSAHs in men. Vulnerability to smoking explains at least in part the paradoxically higher aSAH risk in women observed earlier. All levels of smoking elevate aSAH risk, and reducing or quitting smoking both reduce the risk. Future studies should focus on clarifying whether improving the lipid-profile reduces aSAH risk also in those carrying an unruptured intracranial aneurysm. In addition, they should study the reasons behind women's vulnerability to smoking. Because a severe risk-factor profile elevates sudden-death aSAH risk more than it elevates hospitalized aSAH, hospital-based aSAH studies may underestimate the role played by risk factors. Public health policies focusing on reduction in classic cardiovascular risk factors at population level are likely to reduce both hospitalized and sudden-death aSAH incidence.

# Tiivistelmä

## Johdanto

Kohonneen kokonaiskolesterolin ja miessukupuolen on usein kuvattu liittyvän suurentuneeseen sydän- ja verisuonitautiriskiin. Kirjallisuus kuitenkin paradoksaalisesti kuvaa näiden tekijöiden suojaavan sydän- ja verisuonitauteihin kuuluvalla aneurysmaattiselta lukinkalvonalaiselta verenvuodolta (aSAV). Myöskään vaarallisimman, äkkikuolemaan johtavan aSAV:n riskitekijöistä ei ole väestötason julkaisuja. Tämä väitöskirja pyrki selvittämään mainittujen paradoksien taustoja sekä ruumiinavaustietoja hyödyntäen kartoittamaan väestötasolla äkkikuolemaan johtavan aSAV:n riskitekijöitä.

## Menetelmät

Ensimmäisessä osatyössä artikkelien systemaattinen katsaus tunnisti väitöskirjan kannalta tärkeimmät artikkelit ja loi pohjan hypoteesien muodostamiselle. Artikkelien suurten keskinäisten erojen vuoksi katsaus keskittyi Cochrane-verkoston suositusten mukaan laadulliseen tarkasteluun. Tulokset raportoitiin systemaattisten katsausten ja meta-analyysien raportointisuosituksen (PRISMA) mukaisesti. Kohorttitutkimuksissa seurattiin terveydenhuollon rekistereistä 65 521 henkilöä, jotka osallistuivat FINRISK-tutkimukseen vuosien 1972 ja 2014 välillä. Uusi kohortti kerättiin joka viides vuosi, ja koko seurannan aikana tunnistettiin 543 aSAV:a, joista 98 johti äkkikuolemaan. Äkkikuolemaan johtaneet aSAV:t tunnistettiin tietokonetomografialla, lumbaalipunktiolla tai ruumiinavaustiedoista. Riskitekijät mitattiin seurannan alussa ja tutkimuksessa huomioitiin sekä aSAV:n tunnetut riskitekijät, että mahdolliset sekoittavat tekijät. Puuttuvat riskitekijätiedot täydennettiin tarvittaessa moni-imputaatiomenetelmällä. Aineiston analyysissä käytettiin Coxin mallia, josta johdettiin riskitekijöiden hasardisuhde-estimaatit, väestön riskiosuus sekä multiplikatiiviset ja additiiviset efektin muunnokset, joilla tutkittiin riskitekijöiden vaikutuksen eroa miesten ja naisten välillä. Pitkän seuranta-ajan vuoksi analyysijä täydennettiin kilpailevien riskien mallilla, joka huomioi muista kuin aSAV:sta johtuneet kuolemat seurannan aikana.



## Tulokset

Systemaattisessa katsauksessa havaittiin, että suuren harhariskin tutkimukset raportoivat aSAV:n riskitekijäksi etupäässä matalan kolesterolin, kun taas pienen harhariskin tutkimukset raportoivat riskitekijäksi korkean kolesterolin. Kohorttitutkimuksessa, jossa tarkasteltiin kattavasti lipidiprofilia, havaittiin että epäedullinen lipidiprofiili (korkea LDL ja matala HDL) on merkittävä aSAV:n riskitekijä etenkin miehillä. Tupakointimäärällä oli lineaarinen yhteys aSAV:in ilmaantuvuuteen sekä miehillä että naisilla ja riski lisääntyi mitä enemmän osallistujat polttivat, kun taas tupakoinnin lopettaneilla aSAV-riski oli seurannan aikana pienempi kuin tupakoijilla. Lisäksi havaittiin, että tupakointi lisäsi naisten aSAV-riskiä enemmän kuin miehillä, eikä tupakoimattomien naisten aSAV-riski ollut koholla tupakoimattomiin miehiin verrattuna. Tämä viittaisi siihen, että sukupuoli muuntaa tupakan ja aSAV:n välistä yhteyttä ja että tämä yhteys on voimakkaampi naisilla. Lisäksi tupakointi ja korkea verenpaine lisäsivät äkkikuolemaan johtavaa aSAV-riskiä enemmän kuin sairaalahoitoon johtavaa aSAV-riskiä.

## Johtopäätökset

Aiemmin havaittu paradoksaalinen yhteys matalan kolesterolin ja aSAV:n välillä selittyy ainakin osittain aikaisempien tutkimusten puutteellisilla tutkimusmenetelmillä. Epäedullinen lipidiprofiili lisäsi aSAV-riskiä sekä miehillä että naisilla ja etenkin miehillä sen riskiosuus väestössä oli huomattava. Tulevien tutkimusten tulisi selvittää, voidaanko epäedullista lipidiprofilia parantamalla vähentää aSAV-riskiä myös potilailla, joilla aneurysma on jo todettu. Naisten herkkyyys tupakan haitoille selittää ainakin osittain aiemmissa tutkimuksissa havaitun naisten suurentuneen aSAV-riskin. Jatkotutkimusten tulisi kartoittaa syytä miksi tupakka lisää naisten aSAV-riskiä. Koska epäedullinen riskitekijäprofiili lisää äkkikuolemaan johtavan aSAV:n riskiä enemmän kuin sairaalaan johtavan aSAV:n riskiä, saattavat vain sairaala-aineiston sisältävät tutkimukset aliarvioida riskitekijöiden roolin. Kansanterveyspoliittiset päätökset, jotka johtavat klassisten sydän- ja verisuonitautien riskitekijöiden vähenemiseen väestötasolla, johtavat todennäköisesti niin äkkikuolemaan kuin sairaalahoitoon johtavan aSAV:n esiintyvyyden pienenemiseen.

# Original publications

This thesis stems from following original publications:

- I. Lindbohm JV, Kaprio J, Korja M. Cholesterol as a risk factor for subarachnoid hemorrhage: A systematic review. *PLOS ONE*. 2016;11(4):e0152568.
- II. Lindbohm JV, Korja M, Jousilahti P, Salomaa V, Kaprio J. Adverse lipid profile elevates risk for subarachnoid hemorrhage: A prospective population-based cohort study. *Atherosclerosis* 2018 Jul;274:112-119.
- III. Lindbohm JV, Kaprio J, Jousilahti P, Salomaa V, Korja M. Sex, smoking, and risk for subarachnoid hemorrhage. *Stroke*. 2016;47(8):1975-1981.
- IV. Lindbohm JV, Kaprio J, Jousilahti P, Salomaa V, Korja M. Risk factors of sudden death from subarachnoid hemorrhage. *Stroke*. 2017 Sep;48(9):2399-2404.

The publications are referred to in the text by their roman numerals and are reprinted here with publishers permission.

# Abbreviations

ApoA1	Apolipoprotein A1
ApoB	Apolipoprotein B
aSAH	Aneurysmal subarachnoid haemorrhage
BMI	Body mass index
CI	Confidence interval
CPD	Cigarettes per day
CT	Computer tomography
HDL-C	High-density lipoprotein cholesterol
HR	Hazard ratio
ICD	International Classification of Diseases
LDL-C	Low density lipoprotein cholesterol
MI	Myocardial infarction
PAF	Population attributable fraction
RR	Risk ratio
SBP	Systolic blood pressure
SES	Socio-economic status
TC	Total cholesterol
TG	Triglycerides
UIA	Unruptured intracranial aneurysm

# 1 Introduction

Aneurysmal subarachnoid haemorrhage (aSAH), the third most common stroke type, comprises 3% to 7% of all strokes.<sup>1</sup> As aSAH is, however, the most severe stroke type, the potential life-years of which it deprives a patient are similar to those of the most common type, ischemic stroke,<sup>2</sup> which causes 67% to 82% of all strokes.<sup>1,3</sup> Moreover, aSAH is also the most fatal stroke type; despite advances in invasive treatments, it still kills nearly 40% of those affected and leaves many survivors permanently disabled.<sup>4</sup> It is also a very insidious disease: around 25% of incidental-aSAH patients die suddenly before being admitted to a hospital ward.<sup>5</sup> Because aSAH affects participants younger than those with other stroke types,<sup>2,4</sup> these sudden deaths often occur in asymptomatic and working-age individuals with no history of detected unruptured intracranial aneurysms (UIAs) or cardiovascular disease. Most aneurysms do not rupture, however, and invasive treatments of UIA and aSAH carry considerable risk for morbidity and mortality.<sup>6</sup> These factors complicate UIA treatment decisions and emphasize the importance of identifying high-aSAH-risk subgroups and targeting invasive preventive measures for these individuals only.

In cardiovascular diseases, high total cholesterol (TC) and male sex usually associate with elevated risk. However, a number of aSAHs studies report paradoxically that low TC<sup>7-13</sup> and female sex<sup>14-16</sup> elevate aSAH risk. Currently, the well-established risk factors for aSAHs include increasing age, female sex, hypertension, and smoking,<sup>17,18</sup> whereas studies on other traditional cardiovascular risk factors such as adverse lipid profile,<sup>7-16,19-29</sup> and physical inactivity<sup>30-36</sup> are conflicting and limited. In addition, no studies describe the association of smoking patterns in detail, and causes for elevated aSAH risk among women are yet to emerge. Additionally, to the best of the authors' knowledge, no population-based studies exist on risk factors of sudden death from aSAH, even though, of all aSAHs, they comprise around 25%.<sup>5</sup> This suggests that hospital-based studies not including sudden-death aSAHs may underestimate aSAH risk-factor estimates and nationwide aSAH incidences because of selection bias caused by exclusion of the sudden-death aSAHs. Because the strongest evidence suggests that preventable risk factors play the main role in aSAH development,<sup>37,38</sup> this thesis focuses mainly on the interplay between these risk factors and aSAH. Specifically, this thesis aims to unfold

the lipid- and sex paradoxes in aSAH literature and describe the risk factors for aSAH in a population-based study by also using autopsy records of those who died suddenly outside hospitals.

## 2 Literature review

### 2.1 Pathophysiology of UIAs and aSAHs

One way to classify intracranial aneurysms is to divide them into categories such as saccular, fusiform, dissecting (blood blister-like), mycotic, oncotic, and traumatic.<sup>39</sup> Mycotic, oncotic, and traumatic aneurysms are secondary and result from infection, tumour, or trauma, whereas the fusiform aneurysm is rare and possibly has a distinct aetiology.<sup>39</sup> This thesis focuses on aSAH resulting from saccular UIAs (referred to here as UIA), which are not secondary to a known cause and are the most common aneurysms, accounting for some 85% of all aSAHs.<sup>39</sup> UIAs are mostly acquired degenerative dynamic lesions that may or may not grow and progress to aSAH depending on the individual risk-factor profile.<sup>40</sup> Only a small fraction of UIAs are congenital and are related for example to connective tissue disorders.<sup>40</sup>

UIA is a focal outpouching sac with a distinct neck that most often stems from artery bifurcations, usually near the circle of Willis.<sup>39</sup> Approximately 90% of UIAs arise in the anterior and 10% in the posterior cerebrovascular circulation.<sup>41-43</sup> Outpouching begins when haemodynamic stress, usually aggravated by hypertension, exceeds the tensile strength in vessel walls.<sup>44</sup> The tensile strength of vessel walls decreases because of a complex interplay between inflammation, proteolytic enzymes, vasa vasorum hypoxia, toxins, and altered nitric oxide production, each related to aSAH risk factors.<sup>44-46</sup> This interplay can disrupt mural-cell function in vessel walls and offset the balance between processes of repair and degradation,<sup>44-46</sup> and thus make vessel walls more vulnerable to haemodynamic stress. As UIA individuals age, their UIAs may or may not grow depending on the balance between these processes of repair and degradation in vessel walls. Through these mechanisms, risk factors such as smoking and hypertension can directly contribute to UIA formation and its rupture, which causes aSAH.<sup>46</sup>

### 2.2 Clinical presentation, diagnosis and treatment of aSAH

In aSAH, the UIA bursts and releases blood into the subarachnoid space. Classical symptoms of aSAH include nausea, sudden and severe headache,

eye sensitivity to light, neck stiffness, decreasing cognition, and loss of consciousness.<sup>39</sup> However, presentation of aSAH is insidious and ranges from isolated headache to sudden death.<sup>39</sup> An aSAH may also cause focal neurological symptoms if the aneurysms bleeds into the brain parenchyma or compresses cranial nerves.<sup>39</sup> Roughly 80 to 90% of aSAHs bleed from the anterior circulation, and the other 10 to 20% from the posterior circulation.<sup>47-49</sup> However, these reported ratios may change over time because of more frequent and accurate imaging studies that detect more and smaller aneurysms and because of better survival and higher age of aSAH patients.<sup>50</sup>

aSAH cannot be diagnosed reliably with symptoms alone, and currently aSAH diagnosis relies on computer tomography imaging (CT). The increasing availability of CT in hospitals since the 1970s has led to better diagnostic accuracy of aSAH.<sup>51</sup> Modern CT devices are reliable in aSAH detection and have a positive predictive value between 0.95 and 1.00 in symptomatic patients if CT is conducted within 6 hours of aSAH.<sup>39,51</sup> However, five days or two weeks after aSAH only 85% and 30% of aSAH patients have blood in the subarachnoidal space that can be detectable with CT.<sup>18</sup> If over six hours have passed from the beginning of symptoms, and CT is negative, a spinal tap is necessary to detect xanthochromia in spinal fluid that may result from aSAH.<sup>18,39</sup> This can reveal aSAH up to 2 to 3 weeks after the onset.<sup>18</sup> Cranial vasculature, location, and the anatomy of the aneurysm can be then further studied most reliably with digital subtraction angiography.<sup>17,18,39</sup>

UIAs and ruptured aneurysms are commonly treated with neurosurgical clipping in which a metal clip is placed around the aneurysm neck or with endovascular coiling in which metal coils are inserted into the aneurysm sack.<sup>17,18,39</sup> The purpose of both these methods is to isolate the rupture-prone part of an aneurysm from the circulation. The choice between the two methods is influenced by patient and aneurysm characteristics and by the technical expertise of the treating hospital.<sup>17,18,39</sup> Since the late 1960s, developments in microsurgery and since the 1990s, developments in endovascular treatment of aSAHs have improved its prognosis considerably,<sup>52</sup> and nowadays approximately one of every three aSAH patients can return to work.<sup>53</sup>

## **2.3 Economic impact of aSAH**

Because aSAH is the most severe stroke type and occurs at a younger age

than do other strokes, it causes a considerable social and economic burden.<sup>2,4</sup> Nowadays, aSAH leaves approximately 40% of those affected dead, with many survivors left permanently disabled.<sup>4</sup> The potential life-years of which aSAH deprives victims are comparable to the figure for ischemic stroke, which is roughly ten-fold more common but less severe.<sup>1,2</sup> Studies on direct and indirect costs of aSAH are few; however, one German study estimated that within the first 12 months, an average hospitalized aSAH patient accounts for a total of 38 000 € in direct and indirect costs. This cost depended on aSAH severity, and the German study reported that they excluded the most severely affected and costly aSAH patients. Based on their results, these authors estimated that the annual cost of all new aSAHs was 437 million euros in Germany.<sup>4</sup> If we assume similar aSAH incidence and costs per patient, the very conservative estimate of new aSAHs per year in Finland would be roughly around 30 million euros. However, this estimate excludes most severe aSAH cases. Moreover, long-term aSAH retirement and rehabilitation costs also remain high.<sup>54</sup>

## **2.4 Prevalence of UIA and predictors of their rupture risk**

The estimated prevalence of UIAs ranges from 2% to 3%, but for certain individuals including women and those with detectable atherosclerosis, may be higher around 6%.<sup>17,18,41,55</sup> Imaging studies nowadays often recognize non-symptomatic aneurysms incidentally. Because the average annual rupture rate for aneurysms is only around 1.5%,<sup>42,47</sup> preventive treatments should target individuals at high risk for aSAH if the aneurysms do not cause symptoms. However, the patient and aneurysm characteristics leading to aneurysm rupture remain controversial.<sup>56</sup> The largest multicentre studies performed, the International Study of Unruptured Intracranial Aneurysms<sup>57</sup> and the Unruptured Intracranial Aneurysm Study<sup>48</sup> reveal that large aneurysms and aneurysms located in an anterior communicating artery, posterior communicating artery, or in posterior arteries predict the higher rupture risk. Additionally the studies reported that small (<7 mm) aneurysms are unlikely to rupture contrary to earlier evidence.<sup>56</sup> However, many high-risk patients in these studies<sup>48,57</sup> underwent a surgical or endovascular aneurysm operation, which introduced a major selection bias that emphasized the role played by aneurysm size and reduced associations between risk factors and UIA rupture. In data-analysis, the studies<sup>48,57</sup> used the Cox proportional hazards model, which assumes that the factors causing censoring do not relate to the outcome studied – an assumption



certainly not valid when most censoring is caused by high-aSAH-risk UIA operations. Additionally, the studies<sup>48,57</sup> lacked blood-pressure measurement (a major aSAH risk factor), had a short follow-up, and had a low number of aSAHs; these further limit the reliability of their risk-factor estimates. An alarming indicator of potentially major bias was that when these studies were combined and reanalysed, a protective association between smoking (the most important aSAH risk factor) and UIA rupture risk emerged.<sup>47</sup>

The natural-history study of unruptured aneurysms from the Helsinki University Central Hospital<sup>58</sup> is, to the best of the authors' knowledge, the only study free of selection bias originating in treatment of high-risk UIA individuals, because it stems from an era before operative UIA management in Finland. Its cohort was collected between 1956 and 1978 and includes mainly individuals who had aSAH and multiple UIAs at baseline and whose unruptured UIAs were followed until death or aSAH. This cohort is clearly not an ideal population-based sample but can provide valuable information on the natural history of UIAs. Other advantages when compared to other studies in the cohort<sup>58</sup> are pre-second-aSAH risk factors, a long follow-up, and repeated aneurysm imaging, which also allowed aneurysm growth analysis. Based on studies using this cohort, smoking, increasing aneurysm size, and aneurysm location in the anterior communicating artery elevate UIA rupture risk.<sup>59</sup> In line with the least-biased epidemiological aSAH-risk factor studies,<sup>14-16</sup> studies using this cohort also emphasize - in development of aSAH - the roles played by risk factors in addition to aneurysm size.<sup>42,59</sup> Thus, in addition to patient and aneurysm characteristics, current European and American guidelines on management of UIAs recommend - in addition to UIA size - also considering SBP and smoking in UIA treatment decisions.<sup>17,18</sup> However, routine screening for UIA is currently considered only for individuals with  $\geq 2$  family members with UIA or aSAH.<sup>17,18</sup> This screening strategy has only a minor effect on aSAH incidence at population level and no effective risk-factor based UIA-screening strategy yet exists.<sup>17,18</sup>

Because studies on UIA individuals include only a few aSAHs, and nowadays many high-risk UIA individuals are treated during follow-up, population-based aSAH risk factor studies may offer the only way to reliably study risk factors for UIA rupture. The inefficiency of current UIA screening strategies and the ability to link electronic health records to cohort studies make population-based aSAH studies less prone to selection bias and offer considerably larger sample size. Thus, even in the absence of a UIA cohort, in

countries where aSAH diagnosis is reliable, population-based aSAH studies have the potential to enhance knowledge of factors leading to UIA rupture, because an aSAH results, by definition, from rupture of an UIA.<sup>17,18</sup>

## 2.5 Incidence of aSAH

The generally accepted worldwide incidence of aSAH, derived mainly from studies done in high-income countries, is around 9.0/100 000.<sup>17,18</sup> In middle- and low-income countries reliable estimates of this incidence are hard to collect because of a lack of resources needed to detect and diagnose aSAH individuals.<sup>60</sup> Consequently, studies from these countries report both higher and lower aSAH incidence than those of high-income countries.<sup>1,60</sup> However, if we assume a worldwide aSAH incidence of 9.0/100 000, 660 000 individuals worldwide will suffer from aSAH every year.<sup>60</sup>

aSAH incidence, however, is not uniform and can differ between and within countries. Small non-population-based, and non-nationwide studies have reported that in Finland and Japan aSAH incidence is two-fold that of other countries.<sup>61</sup> However, large nationwide population-based studies<sup>5,62,63</sup> have recently questioned these findings and showed that aSAH incidence varies considerably among different areas and age groups within one nation. These same studies<sup>5,62,63</sup> indicate that incidence estimates derived from small population subgroups may only reflect regional aSAH incidence variation; as nationwide incidence estimates, they are not valid. These studies may, however, serve to detect regional high-aSAH-risk subgroups. Apparently only Finnish, Swedish, Danish, and Norwegian studies<sup>64-67</sup> include in their incidence estimates also sudden-death aSAHs (about 25% of all aSAHs). These population-based nationwide studies report fairly similar aSAH incidences of 8.9 to 12.0/100 000.<sup>64-66</sup> Large population-based studies from other industrialized countries provide similar aSAH incidence estimates which range from 8.0 to 13.7/100 000.<sup>68-70</sup> This indicates that, contrary to common belief aSAH incidence is not exceptionally high in Finland and suggest that nationwide Finnish aSAH studies may have fairly good external validity, and their results may be generalizable at least to other Nordic countries, and possibly more generally to populations of European ancestry.

The incidence estimates may also change over time. Whereas stroke incidence has been reduced during the last 30 years in high-income

countries<sup>1</sup> aSAH incidence has been more stable in studies conducted before 2000.<sup>1,61</sup> The reasons behind this finding are unclear but better ability to differentiate aSAH from other haemorrhagic strokes by means of improved quality of CT imaging may have played a role. After 2000, studies from Finland, Norway, Sweden, and Denmark report a between 11% and 24% decrease in aSAH incidence standardized for the European population. Between 1998 and 2012, aSAH incidence in Finland has fallen 24% from 11.7/100 000 to 8.9/100 000.<sup>5</sup> During the same interval, smoking prevalence has decreased roughly 9%, and mean SBP and TC have decreased roughly 3% and 5% in Finland.<sup>71</sup> Data from other countries with reliable aSAH incidence estimates also suggest similar trends. In Norway, between 1999 and 2007, aSAH incidence has decreased 20% from 11.1/100 000 to 8.9/100 000,<sup>64</sup> and during this period the reductions in smoking prevalence, in mean SBP, and in TC have been around 13%, 5%, and 7%.<sup>72,73</sup> Nationwide studies from Sweden and Denmark report around 11% reductions in aSAH incidence, but these studies<sup>65,67</sup> are older than the Finnish and Norwegian studies, which may in part explain the smaller reduction.

The decrease in aSAH risk factors has occurred simultaneously with an increase in mean aSAH age. Studies conducted in the 80's and 90's report that aSAH incidence peaks around age 60,<sup>74</sup> whereas studies after 2000 from Norway and Finland describe a peak around age 70.<sup>5,64</sup> aSAH incidence is higher in men before age 55, but after this, the incidence is higher in women.<sup>7,15,61</sup> The main hypothesis is that menopause relates to higher aSAH rates in women through their decreased oestrogen levels,<sup>45,75</sup> but this hypothesis remains untested in female humans. In general, aSAH incidence is low (< 2/100 000) before age 30 but then rises steadily until 70 to 75.<sup>64-66</sup> After this, the incidence seems to somewhat decrease, possibly because individuals who survive to this age may have fewer aSAH risk factors. No new aSAH risk factors have emerged since the 80's because current guidelines recognize only increasing age, hypertension, smoking, and female sex.<sup>17,18</sup>

## **2.6 Risk factors for aSAH**

When studying aSAH risk factors, one concern is the potential bias resulting from exclusion of the ~25% of aSAH individuals who die suddenly outside hospitals. This exclusion may bias the risk-factor estimates if the risk factors or

their associations in these individuals differ from that of hospital-based aSAH individuals. Therefore, when possible, this section focuses on studies that have included also sudden-death aSAHs.

### **2.6.1 Age**

Epidemiologically, age is a measure of time since birth and thus per se is not a risk factor, whereas ageing refers to the cumulative effects of risk factors. Because of this, however, calendar age is a major confounder because it relates to many risk factors and their cumulative effect. Although population-based prospective studies describe increasing aSAH incidence as individuals age,<sup>64-67</sup> this may at least in part reflect the cumulative effect of residual confounding from other aSAH risk factors. These are factors that may lead to development of aneurysms through inflammation and wall-shear stress, which slowly leads to a damaged vessel wall, and to formation of rupture-prone aneurysms as individuals age.<sup>44</sup>

### **2.6.2 Sex**

Epidemiologically, sex is also not an independent risk factor, but rather a proxy for biological differences between men and women. Studies consistently report elevated aSAH risk in women with HR ranging from 1.4 to 2.1,<sup>7,16,76</sup> but the reasons behind the higher risk are still unclear. Effect estimates of some studies imply that hypertension and smoking can show differing association in women than in men<sup>14-16</sup> but no well-powered studies have systematically studied this potential effect modification. One widely speculated theory is that the higher risk relates to changes in hormonal action and a subsequent decrease in oestrogen levels<sup>75</sup> Supporting this theory is the observation that aSAH risk becomes higher in women post-menopausally after age 55.<sup>7,15,61</sup> Another explanation for the sex difference may be survival bias relating to the worse risk-factor profile in men that could lead to higher numbers of unrecognized sudden-death aSAH in men than in women. However, this is unlikely because studies from Nordic countries that include also sudden-death aSAH report similar HR estimates for female sex<sup>16</sup> as do other studies.<sup>76</sup> Thus the reasons behind the paradoxical association between female sex and aSAH remain to be studied.

### **2.6.3 Smoking**

The data on smoking and aSAH are rather consistent, with many prospective

studies reporting that smoking elevates aSAH risk with a HR between 2.2 and 5.7<sup>7,15,16,77</sup> – a very strong association in the epidemiological context. However, these estimates are derived from current smokers, and because smoking rates decrease during follow-up,<sup>78</sup> these baseline risk estimates are potentially underestimations. In addition, studies usually model smoking only as a binary variable, meaning that light and heavy smokers are included in the same category.<sup>7,15,16,77</sup> This leads to an imprecise variable that potentially carries considerable residual confounding that can mask a potential effect modification or interactions between other variables and smoking. Moreover, estimates of cumulative smoking, measured for example in pack-years, ones that could capture life-time- and dose-response exposure to smoking and allow more detailed risk modelling, are few. Two retrospective case-control studies reported that smoking cessation reduces aSAH risk, but the risk may not be lowered among heavy smokers who quit.<sup>79,80</sup> This would suggest that a critical limit for pack-years exists, and after exceeding this limit quitting smoking is insufficient to reduce aSAH risk. However, because these studies used only the binary variables of hypertension and hyperlipidemia, their estimates may at least in part reflect residual confounding if former smokers had more severe and long-term hypertension or hyperlipidemia. Thus, the data on association of smoking cessation and smoking habits with aSAH are still limited, and studies on dose response and effects of aSAH in different population subgroups are few.

The strong association also has biological support, and the causal pathway from smoking to aSAH is thought to relate to every process of aneurysm formation.<sup>44-46</sup> Smoking elevates wall shear stress by elevating blood pressure, and it causes increased blood viscosity. Inhaled toxins cause systemic inflammation, which is followed by migration of neutrophils and by release of elastase that each further causes vessel walls to be more vulnerable to wall shear stress.<sup>44-46</sup> In women, smoking also has the potential to reduce the protective effect of oestrogen.<sup>45</sup> The elimination of these processes after quitting smoking is thus likely to lead to decreased aSAH risk.

#### **2.6.4 Hypertension**

Prospective studies on hypertension and aSAH also consistently report that diagnosed hypertension elevates aSAH risk with an HR between 1.7 and 2.5.<sup>7,15,16,77</sup> However, blood pressure elevates aSAH risk dose-dependently and the risk may be considerably higher with severe untreated hypertension.<sup>7,15,16,77</sup>

This indicates that the categorical hypertension variable is too inaccurate and imports considerable residual confounding into the risk estimates. In addition, antihypertensive medication may lower SBP and thereby reduce aSAH risk,<sup>81,82</sup> supporting the view that modelling of SBP instead of hypertension status may lead to more reliable risk estimates. Moreover, studies on hypertension in different population subgroups are limited, and no long-term prospective studies on effects of antihypertensive therapy on aSAH risk currently exist. During long follow-up, antihypertensive interventions may thus lead to decreased risk estimates for hypertension.

The causal pathway from hypertension to aSAH is thought to relate to increased wall shear stress, endothelial damage, disturbance of smooth muscle cell function in vessel walls, and occlusion of vasa vasorum that leads to hypoxia and necrosis in vessel walls.<sup>44-46</sup> By reducing these processes, hypertension treatment may reduce aSAH risk.

### **2.6.5 Alcohol consumption**

Some case-control studies and cohort studies have found an association between aSAH and alcohol consumption and some have not.<sup>7,16,21,77</sup> Although amount of alcohol consumed and background characteristics in these studies have varied, most studies report elevated aSAH risk among heavy drinkers (>150 g of pure alcohol per week).<sup>7,16,21,77</sup> The prevailing obstacle in studying associations between alcohol and aSAH is the limited number of heavy-alcohol consumers who do not smoke. This limits the ability to differentiate between the effects of alcohol and smoking; a further complication is underreporting of alcohol consumption.<sup>83</sup> If heavy alcohol drinking is clustered among heavy smokers who also underreport their alcohol consumption, the HR of smoking may reflect the combined association of smoking and alcohol consumption. Because alcohol consumption may also lead to hypertension and dyslipidemia, these may mediate the association between alcohol and aSAH,<sup>21</sup> however, to the best of authors' knowledge, no mediation studies on this subject exist.

The suggested causal mechanism from heavy alcohol consumption to aSAH include damaged endothelium by elevated oxidative stress and wall shear stress caused by hypertension and elevated blood viscosity.<sup>44,45</sup>

### **2.6.6 Lipid profile**

Studies on TC and risk for aSAH are conflicting, and reports show both

high<sup>15,25,28,29</sup> and low<sup>7,9,11-13</sup> TC to raise risk. Because case-control studies typically measure the lipid profile after aSAH, they are prone to reverse causation if the lipid profile does not reflect long-term lipid profile due to medication or if the lipid-profile changes during or after aSAH. Additionally, most case-control studies omit data on individuals' previous risk-factor levels and their medications, which further complicates interpretation of their findings. Cohort studies may be unable to control changes in lipid values during follow-up, and an adverse lipid profile in the beginning of follow-up often leads to treatment. During follow-up, this inability to control for treatment may lead to underestimation of the association between lipid-profile and aSAH, especially because the widely used, most common lipid-lowering medication, statins, may have pleiotropic,<sup>84</sup> vasculature-protective effects and can even reverse atherosclerosis.<sup>85</sup>

Meta-analyses have found no association between TC and aSAH, and suggest that high HDL-C may protect against aSAH.<sup>77,86,87</sup> These meta-analyses summarize methodologically very different studies which have differing lipid measurement protocols, differing selection of cases and controls, and differing confounder adjustment and data analysis. Additionally, increased statin use after 1993 further complicates interpretation of meta-analyses, because some of the cohorts included are from the pre-statin era, and some from the post-statin era.

Rat models suggest that the protective associations of hypercholesterolemia relate to enhanced cell maintenance and to decreased smooth muscle cell death.<sup>45</sup> However, deleterious associations of hypercholesterolemia also have biological support. Atherosclerosis is commonly observable in aneurysm walls, with histological studies on human aneurysms suggesting that hypercholesterolemia associates with the elevated inflammation and the smooth-muscle cell death that predisposes UIAs to rupture.<sup>88,89</sup> Histological findings of an adverse effect of lipid profile thus lack, paradoxically, support from epidemiological studies.

### **2.6.7 Other risk factors**

Some studies report no association between aSAH and BMI,<sup>15,23,29,90</sup> but most prospective studies show that low BMI elevates aSAH risk.<sup>7,14,16,91,92</sup> The studies on BMI, however, have varying follow-up times, they control confounders differently, and they include different proportions of hospitalized aSAHs and

sudden-death aSAHs, which may in part explain the differences between such studies. In addition, because the excess adiposity associated with high BMI predisposes to adverse lipid profile and hypertension,<sup>93</sup> and to the author's best knowledge, no aSAH studies include mediation analysis of BMI, hypercholesterolemia, and hypertension, the true association and potential independent causal pathways between BMI and aSAH remain unclear.

Prospective studies on diabetes and aSAH are few, but case control studies describe no association or an inverse one.<sup>77,94</sup> On several biological bases, this seems contradictory because diabetes elevates cardiovascular disease risk in general. What makes this finding even more interesting is that early stages of diabetic retinopathy are associated with retinal microaneurysms, which may in part have a pathogenesis similar to that of UIAs.<sup>95</sup> However, the potential inverse associations may be associated with better control of smoking and hypertension as well as to higher rates of competing cardiovascular events related to diabetes.<sup>77</sup>

Leisure-time physical activity associates with reduced risk for ischemic stroke,<sup>35,96</sup> but only a few studies on aSAH and physical activity exist,<sup>30-36</sup> and these present inconsistent results. The physical activity variables used in these studies differ considerably, and some add leisure-time, commuting, and occupational physical activity to overall physical activity, whereas others use only leisure-time physical activity.<sup>30-36</sup> Combining figures for different physical activity groups may be problematic because white-collar workers who are sedentary at work may have better health than blue-collar workers who are physically active at work and achieve high overall physical activity. This fact could in part mask the potential beneficial association between high overall physical activity and aSAH if socio-economic differences are not controlled for. Moreover, some evidence indicates that different physical activity types may associate differently with cardiovascular diseases.<sup>96</sup> In addition, the benefits of physical activity may be mediated via lower rates of hypertension development during follow-up. To the authors' knowledge, no mediation analysis on this subject exists. The potential beneficial causal mechanism among those physically active may also relate to reduced systemic inflammation<sup>97</sup> and to reduced inflammation in intracranial vessel walls.<sup>44</sup>

Since the suggestion of a familial predisposition for aSAH, several studies have sought for genes related to UIAs.<sup>38,98,99</sup> However, associations between genes and aSAH remain modest<sup>38,98,99</sup> and the concept of familial aSAH



remains controversial, especially since studies describing this association have failed to adjust their results for known aSAH risk factors that may also cluster in high-risk families.<sup>37</sup> One large twin study further questioned the concept of familial aSAH by showing that heritability of aSAH is ~40%,<sup>37</sup> which is lower than the heritability of coronary artery disease,<sup>100</sup> (~50%) suggesting that aSAH does not carry an exceptional familial component. Additionally, many genes that elevate cardiovascular disease risk act by elevating traditional risk factors such as LDL-C and SBP,<sup>100,101</sup> further complicating differentiation of direct genetic effects on the disease compared to mediation through risk factors, many of which have both a genetic and an environmental component.<sup>102</sup> In the absence of large studies including repeated risk-factor measurements and of a sufficient number of aSAHs, the potential complex interplay between genes, risk factors, and aSAH remains undescribed. However, individuals with  $\geq 2$  first-degree family members with aSAH do have a high lifetime aSAH risk and may be the only reasonable group for UIA screening, despite current uncertainty regarding any aetiological pathway.<sup>17,18</sup>

Other factors suggested to relate to aSAH are oral contraceptives, hormonal replacement therapy and polycystic kidney disease.<sup>77</sup> Results on oral contraceptives and hormonal replacement therapy are conflicting,<sup>103-105</sup> partly because of unmeasured confounding, residual confounding, and differences in oestrogen and progesterone doses and their ratio. Earlier studies also speculate that polycystic kidney disease may elevate aSAH risk, however, current guidelines do not strongly support this.<sup>17,18</sup>

## **2.7 Common methodological problems**

aSAH risk-factor studies are challenging to conduct for a number of reasons. Often the main issue is the limited sample size that results from low overall incidence and a high outside-hospital death-rate. Because only countries with a high coverage of forensic autopsies are able to detect comprehensively outside-hospital deaths from aSAH, reliable nationwide incidence estimates are rare and available mostly in the Nordic countries.<sup>64-67</sup> Additionally, in aSAH risk-factor studies, exclusion of sudden-death aSAHs presents a potentially considerable selection bias in all case-control studies and in most cohort studies when autopsy records are not available especially if risk factors' associations with hospitalized and sudden-death aSAH differ.

Another problem, especially in case-control studies, arises from differing risk factor measurement methods in case and control groups which can lead to information bias and to reverse causality. For example, because aSAH is a severe disease and causes many physiological responses, in case-control studies, the laboratory measurements performed at a hospital may not correspond to their pre-aSAH values.<sup>106</sup> This has also been observed in studies on acute myocardial infarction (MI).<sup>107</sup> aSAH patients are also often unable to provide reliable questionnaire-based pre-morbid risk-factor estimates because of their death or morbidity, which further lessens the reliability of case-control studies. In addition, those who suffer an aSAH (or their relatives) may become more aware of stroke cases in their families, which may cause recall bias<sup>108</sup> in assessment of familial aSAHs. Additionally, if analyses fail to include commonly used medications such as statins and antihypertensive medication, the risk factor measured after aSAH may merely reflect the result of medication – not the individual's long-term risk-factor profile – and this can lead to misclassification of high-risk individuals at baseline.

In cohort studies, confounding by indication may arise, because those who are high-risk individuals at baseline and during follow-up are more likely to receive health advice and medication. For example, if those with high TC at baseline receive cholesterol-lowering medication, the association between TC and aSAH could be attenuated or even reversed. In addition, few cohort studies can control risk factor changes over time, which may lead to increasing exposure misclassification during follow-up. For example, half of all smokers quit during a long follow-up,<sup>78</sup> which may weaken the association between smoking and aSAH. Moreover, the same risk factors that associate with aSAH are also associated with other cardiovascular diseases, and this can lead to an early end of follow-up because of competing causes of death. In aSAH studies using UIA cohorts, competing causes can also be surgical or endovascular interventions that reduce the risk for aneurysm rupture. This indicates that every aSAH risk-factor study should include a competing risks analysis, and this would be especially useful when studying the rupture risk of UIA cohorts in an era when surgical or endovascular interventions treat the high-rupture-risk patients. In this situation, an aneurysm operation becomes a competing event for aSAH meaning that the same risk factors that lead to an aneurysm operation are also related to aneurysm rupture rate, indicating that results omitting competing risks may be biased.

### 3 Study Aims

This thesis aims to understand the paradoxical associations between TC and aSAH and between female sex and aSAH and additionally aims to characterize risk factors for sudden-death aSAH at population level. Specific aims consist of four goals:

- 1) Clarification of reasons behind the reported opposite associations of TC and aSAH by means of a systematic review.
- 2) Clarification of association between lipid profile and aSAH with a population-based prospective cohort study that includes detailed lipid profile analysis, outside-hospital sudden-death aSAHs, analysis by sex, and pre- and post-statin medication-era analysis.
- 3) Characterization of any association between smoking habits and aSAH in detail, and description of these associations in men and women.
- 4) Characterization of risk factors for sudden-death aSAH by inclusion of data from autopsy records, and comparison of the detected risk factors to those of hospitalized aSAH.

# 4 Methodology

## 4.1 Systematic review and meta-analysis (Study I)

The International Prospective Register of Systematic Reviews; (registration code: CRD42015016347) presents review articles' study protocols, which follows the Preferred Reporting Items for Systematic reviews and Meta-analyses for Protocols Statement. Cochrane Library, Pubmed, and Scopus databases provided the platform for the literature search with no language limitations, and native speakers assisted with non-English publications. We compared the studies identified by the preliminary search to studies listed in their references to spot any differences in indexing or gaps in the preliminary search. Based on limitations in the preliminary search and on aid provided by an information specialist, the final search protocol took form. The population, intervention, comparison, outcome format was: Do low- or high cholesterol or lipoprotein levels associate with risk for aSAH in adults? The reference list of the second search was a platform for indirect validation of the results; we did not identify any new applicable studies from among the references. We ran the search for the final time on 15 December 2015. Although we requested additional data from the authors of 21 studies that had limited data for systematic review, only 2 replied, indicating that individual personal data analysis was impossible.

Study inclusion criteria were: 1) at least two categories for TC, LDL-C, HDL-C, or apolipoprotein concentrations with effect estimates, 2) at least 50 cases of aSAH. Based on Cochrane Collaborations recommendations,<sup>109</sup> we used the non-numerical Critical Appraisal Skills Program<sup>110</sup> and the Cochrane Collaboration Handbook<sup>109</sup> in our risk-of-bias estimates. Our review scrutinized in particular, measurement bias, selection bias, reporting bias, confounder adjustment, reverse causality, and statistical power.

Optimistic sufficient sample size calculations stemmed from the Cox proportional hazards model: standard significance value of  $p < 0.05$ , standard power value of  $P = 0.8$ , correlation factor value of 0.1 between covariates, HR 2.0 (95% CI 1.0-4.0), and an incidence value of 20/100 000. To avoid too-optimistic associations, the random effect model provided  $I^2$  statistic and pooled risk ratios (RR) estimates in inverse variance weight meta-analysis. Population attributable fraction (PAF) estimation was done with following

formula:  $PAF = p_f (RR-1) / [p_f (RR-1) + 1]$ , where  $p_f$  indicates population fraction with hypercholesterolemia and RR relative risk.

## 4.2 The cohort studies

### 4.2.1 FINRISK participants in Studies II-IV

The National FINRISK Surveys, performed every five years since 1972, gathered data on independent, population-based, random samples of adults from various geographical areas of Finland. Surveys in 1972 and 1977 comprised participants from North Karelia and North Savo, both located in eastern Finland.<sup>71</sup> Since 1982, the Turku and Loimaa regions in southwestern Finland were also included, and from 1992 onward, the study comprised also the capital area of Helsinki and Vantaa in southern Finland.<sup>71</sup> The provinces of North Ostrobothnia and Kainuu in northwestern Finland have also provided data since 1997.<sup>71</sup> A 6.6% random sample of the population born between 1913 and 1947 formed the cohort collected between 1972 and 1977. From 1982 to 2002 a random sample of individuals aged between 25 and 65, stratified by sex and 10-year age-group produced the cohorts.<sup>71</sup> Since 2007, the corresponding age-group comprised those aged between 25 and 75. The participation rates were above 90% in the 1970s, have steadily decreased, but are above 60% in the most recent surveys.<sup>71</sup> The study methodology remained comparable in all surveys in order to achieve comparable cohorts.

The population register of Finland provided information about participants' sex and age. A standardized self-administered questionnaire collected data on the following variables: alcohol consumption, history of hypertension, medication for hypertension, medication for hyperlipidemia, physical activity, smoking status, marital status, and socio-economic status (SES) measured by years of education.<sup>71</sup> Local study centres provided facilities for anthropometric measurement including height, weight, semi-fasting blood samples after at least 4-hour fasting, and systolic and diastolic blood pressure.<sup>71</sup> These measurements were performed by trained and experienced study nurses at enrolment. The venous blood samples were centrifuged on measurement sites and the sera were sent daily by mail to the laboratory of the National Public Health Institute for cholesterol measurements.<sup>71</sup> Starting from 2007, the sera were frozen immediately after separation of serum and sent to National

Public Health Institute once a week for cholesterol measurements.<sup>71</sup>

#### **4.2.2 Blood pressure**

Mercury sphygmomanometers measured blood pressure in all surveys, and the cuff bladder size was 13 cm by 23 cm in 1972 and 1977, 13 cm by 42 cm from 1982 to 1997, and was 14 cm by 36 cm from 2002 to 2012.<sup>71</sup> A minimum of 5 minutes of rest preceded the measurements done from the right arm in a seated position. The first phase of Korotkoff sounds determined SBP, and the fifth phase determined diastolic blood pressure.

#### **4.2.3 Smoking**

Participants reporting smoking no more than between 0 and 100 cigarettes in their lifetime were considered never-smokers. Those who had smoked on a non-daily basis during the 6 months prior to enrolment were considered occasional smokers. Those participants who had quit smoking within 6 months before enrolment were recent quitters, whereas those who had quit over 6 months before enrolment were former smokers. Current smokers provided the number of cigarettes smoked per day (CPD) on average separately for cigars, pipefuls of tobacco, and manufactured and self-rolled cigarettes. Because studies suggest that one cigar elevates aSAH risk as much as one cigarette, and one pipeful of tobacco as much as three cigarettes,<sup>111-115</sup> we also included these variables, scaled as cigarettes, into the final CPD variable. Cotinine measurements indicate that the validity of self-reported smoking status is good, whereas cotinine levels and reported amount of smoking among smokers correlate only modestly.<sup>116</sup>

We used eight categories of smoking status in our analyses: never-smokers, occasional smokers, former smokers, recent quitters, and four groups of current smokers. Based on CPD, we divided smokers into 1 to 10, 11 to 20, 21 to 30, and >30 CPD. We calculated pack-years for current smokers by the following formula:  $[(\text{CPD} \times \text{number of years smoked}) / 20]$  and divided them into groups of none (for all but current smokers), <5, 5 to 10, and then in 10 pack-year intervals until >50.

#### **4.2.4 Lipid measurements and lipid lowering drugs**

In 1972 and 1977, the Lieberman Burchard method served for analysis of

serum TC and triglycerides (TG) and from 1982 onwards, an enzymatic method (CHOD-PAP; Boehringer MANNHEIM, Mannheim, Germany)<sup>117</sup> served for performing these the analyses. For all surveys, all pre-analysis procedures were the same.<sup>117,118</sup> Dextran–MgCl<sub>2</sub> precipitation, the enzymatic CHOD-PAP method, served for analysis of HDL-C between 1982 and 1997, and from 1998 onwards, a direct method served for the analyses.<sup>119</sup> A direct method measured also LDL-C, and when LDL-C was calculable, it was also estimated by the Friedewald formula by limiting TG to <4.52 mmol/l. Because we had more LDL-C values, and because the Spearman correlation between directly measured LDL-C and Friedewald LDL-C was 0.96, we chose to use calculated Friedewald LDL-C in our analyses. In addition, the immunoturbidimetric method of Abbott Architect reagents (Abbott Laboratories, Abbott Park, IL, USA) has served for measurement of ApoA1 (major lipoprotein in HDL-C) and ApoB (the primary lipoprotein in chylomicrons, VLDL-C, IDL-C, and LDL-C) since 1992.<sup>120</sup> All values measured in FINRISK are under external quality control in order to avoid systematic errors.<sup>117</sup> Data on lipid-lowering drugs were collected by self-administered questionnaires.

#### **4.2.5 Alcohol consumption**

The self-administered questionnaire collected data on the amount of alcohol consumed during the preceding seven days. The amount has been reported for each generally available alcohol type in Finland, including beer, wine, long drink, and hard liquor, since 1982. In 1972 and 1977, questionnaire-collected data involved only on beer and wine as well as total alcohol consumption. For analyses, we scaled the alcohol variable as absolute alcohol grams consumed per day.

#### **4.2.6 BMI**

Study nurses measured lightly clothed participants' weight with a beam balance scale and rounded the result to the nearest 100 grams. In 1972 and 1977, height was rounded to the nearest 0.5 cm, whereas from 2002 onwards it was rounded to the nearest 0.1 cm.<sup>71</sup> In our analyses, weight in kilograms divided by squared height in meters yields BMI.

#### **4.2.7 Diabetes**

Baseline diabetes diagnoses were recorded by self-administered questionnaire

at enrolment, and during follow-up, the National Drug Reimbursement Register provided data on incident diabetes diagnoses. We had these data available for the study on lipid profile and aSAH only.

#### **4.2.8 Education and marital status**

Participants reported their years of education (used as a proxy for SES), which were then scaled to tertiles within each survey year because of changes in education duration during follow-up. Participants also reported their marital status in four categories: 1) married or cohabiting, 2) single, 3) divorced, 4) widowed. For analyses, we transformed marital status also into two categories: 1) married or cohabiting, 2) without a partner (single, divorced, or widowed).

#### **4.2.9 aSAH identification and definition**

Enrolment marked the beginning of follow-up which ended at first-ever aSAH, death, or on December 31, 2014, whichever came first. For Study III, the data covered follow-up only until December 31, 2011. For all participants who remained in Finland, the follow-up was complete, emigration during follow-up being rare.<sup>1</sup> The nationwide Hospital Discharge Register and Causes of Death Register collected nonfatal and fatal aSAHs with high accuracy, covering also outside-hospital and emergency-room aSAH deaths.<sup>22</sup> From these registries, we retrieved incident aSAHs by using International Classification of Diseases (ICD), codes 430 (in ICD-8 and ICD-9 classification) or I60 (in ICD-10 classification). aSAH individuals who reached hospital were diagnosed mainly with CT and/or spinal tap, whereas in addition to these methods sudden-death aSAH diagnosis was confirmed with forensic autopsy or medical autopsy when the incidents occurred away from a hospital, in an ambulance, or in an emergency room. For all sudden and unexpected deaths outside hospital in Finland, a forensic autopsy is mandatory. A nosologist at Statistics Finland examines all death certificates and, if needed, rectifies the underlying cause of death. The diagnosis protocol described yielded a positive predictive value of 87% for aSAH.<sup>121</sup> The Strengthening Reporting of Observational studies in Epidemiology statement<sup>23</sup> steers the reporting of all studies included.



## 4.3 Statistical methods

### 4.3.1 Missing data and imputations

For values of ApoA1, ApoB, HDL-C, and LDL-C, the missing at random assumption<sup>122</sup> applied, because study year alone explained missingness. For variables included in the imputation model, the percentages of missing values were: for smoking status 1.1%, SBP 1.6%, TC 1.9%, HDL-C 36.1%, TG 41.1%, LDL-C 58.8%, ApoB 65.5%, and ApoA1 65.8%. Continuous variables in the imputation model were ApoA1, ApoB, BMI, HDL-C, LDL-C, SBP, and TC, whereas the categorical variables included were quartiles of alcohol consumption, eight categories of smoking, three categories of SES (measured as years of education), and use of a cholesterol-lowering drug as a binary variable. Linear regression imputed continuous-, ordered logistic regression imputed ordinal-, and logistic regression imputed binary variables. The imputation model included also an effect modification term between smoking and sex, because smoking associates with aSAH more strongly in women.<sup>123</sup> Our imputation model additionally allowed for interaction or effect modification between each lipoprotein variable and sex, hypertension, or smoking. Substantive-model-compatible fully conditional specification formed the basis of the imputation model because it handles nonlinear and effect modification models better than does the ordinary fully conditional model.<sup>124</sup> Our analysis used from 50 to 150 iterations depending on convergence and 80 imputations to minimize simulation error. Based on imputation convergence analyses,<sup>125</sup> our imputation model reached statistical reproducibility with sufficient iterations. A pilot analysis in which 10%, 30%, or 60% of the observed HDL-C, LDL-C, ApoB, or for ApoA1 values were first deleted and then imputed back showed that our model performed well even when 60% of data was missing.

### 4.3.2 Analysis models

Students t-test calculated p-values for variables with normal distribution, while a Wilcoxon-rank test and chi square test calculated p-values for skewed and categorical variables. For unadjusted and adjusted models, the Cox proportional hazard model provided hazard ratios (HRs) and 95% confidence interval (CI). The competing risks model<sup>126</sup> calculated the associations in the presence of other causes of death during the long follow-up. When comparing differences in risk factors between sudden-death aSAH and hospitalized aSAHs, we compared the cause-

specific hazard functions by risk-factor status. We performed these analyses by sex and previously defined smoking categories and when possible used continuous variables. After reviewing prospective and population-based studies,<sup>7,15,16</sup> we chose age, sex, smoking, SBP, and TC (or because of collinearity one of the following: HDL-C, LDL-C, ApoA1, or ApoB) to put into our final model. Additionally, the final model included BMI, study year, and study area as possible confounders and to control for cohort effect. We also included self-reported cholesterol-lowering-drug use, alcohol consumption, and years of education as proxy for SES into preliminary models. Between our variables and aSAH, we searched for non-linear associations with cubic splines and multivariable fractional polynomial models.<sup>127</sup> Inverse of HDL-C ( $\text{HDL-C}^{-1}$ ) associated more strongly with aSAH than did HDL-C, and was thus included in our final analysis model for HDL-C. Schoenfeld residuals and log-log plot inspection showed that proportional assumption criteria applied in our models. A likelihood ratio test tested multiplicative interactions or effect modifications, whereas an additive interactions method<sup>128</sup> provided relative excess risk due to interaction, attributable proportion, and synergy index in our adjusted models. A method restricting overall PAFs to 100%<sup>129</sup> provided population-attributable fractions (PAFs) of the cases preventable by eradication of a risk factor. Stata Corp version 12.1 (College Station, TX, USA), and R 3.3.0 served to perform all analyses.

## 5 Ethics statement

The local ethics committees gave approval for each FINRISK survey in accordance with legislation pertinent to the time of the survey. The World Medical Association's Declaration of Helsinki on ethical principles for medical research also guided the studies. Each participant provided oral informed consent between 1972 and 1997, whereas from 2002 onwards, participants also gave a written informed consent.<sup>130</sup>

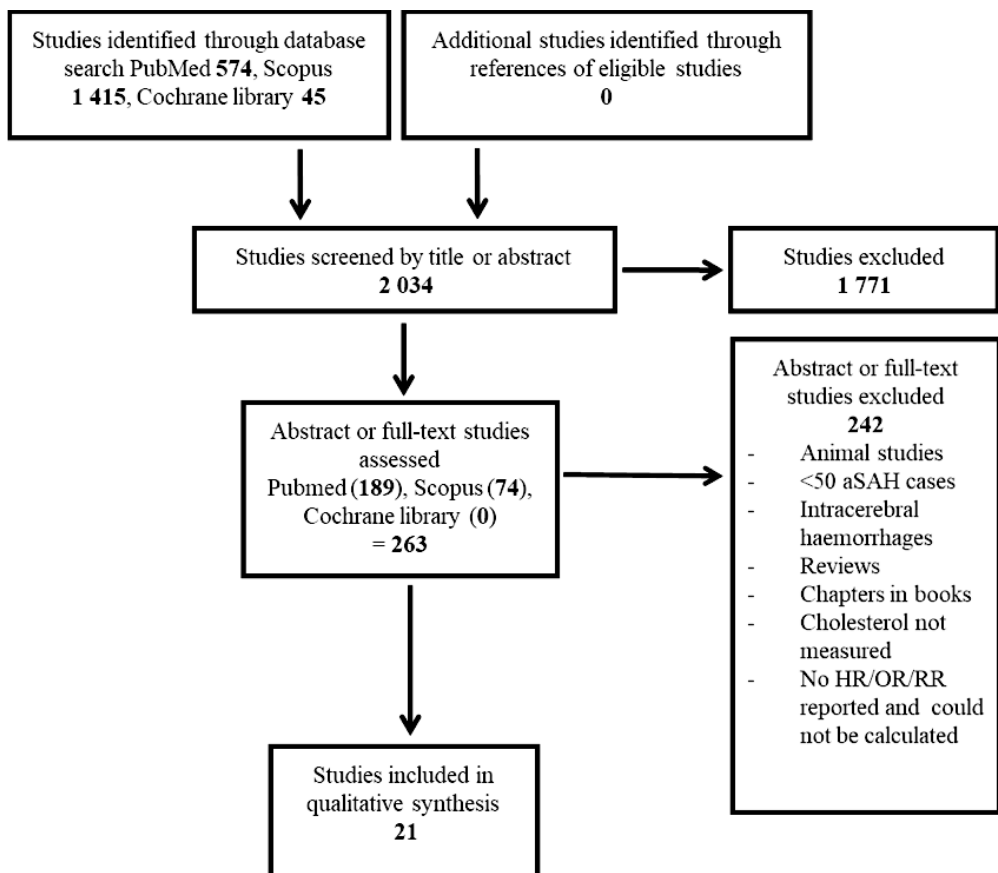
## 6 Results

### 6.1 Review on lipid profile and aSAH (Study I)

#### 6.1.1 Studies identified and reviewed

Of the 21 studies <sup>7-16,19-29</sup> that met our inclusion criteria (Figure 1), 12 were prospective and 9 retrospective. Of the prospective studies, six originated from

**Figure 1.** Flowchart describing how studies were selected for systematic review (Study I).



Adapted from *PLOS ONE*. 2016;11(4):e0152568, Lindbohm et al., “Cholesterol as a risk factor for subarachnoid hemorrhage: A systematic review,” by permission from Public Library of Science.

Nordic countries (four from Finland, one from Norway, one from Sweden), three from the USA, two from Japan, and one from South Korea.<sup>7,13-16,19-25</sup> Of the retrospective studies, four came from Japan and one each from the following countries: the USA, Portugal, South Korea, The Netherlands, and from a Denmark- and Great Britain-based collaboration.<sup>8-12,26-29</sup>

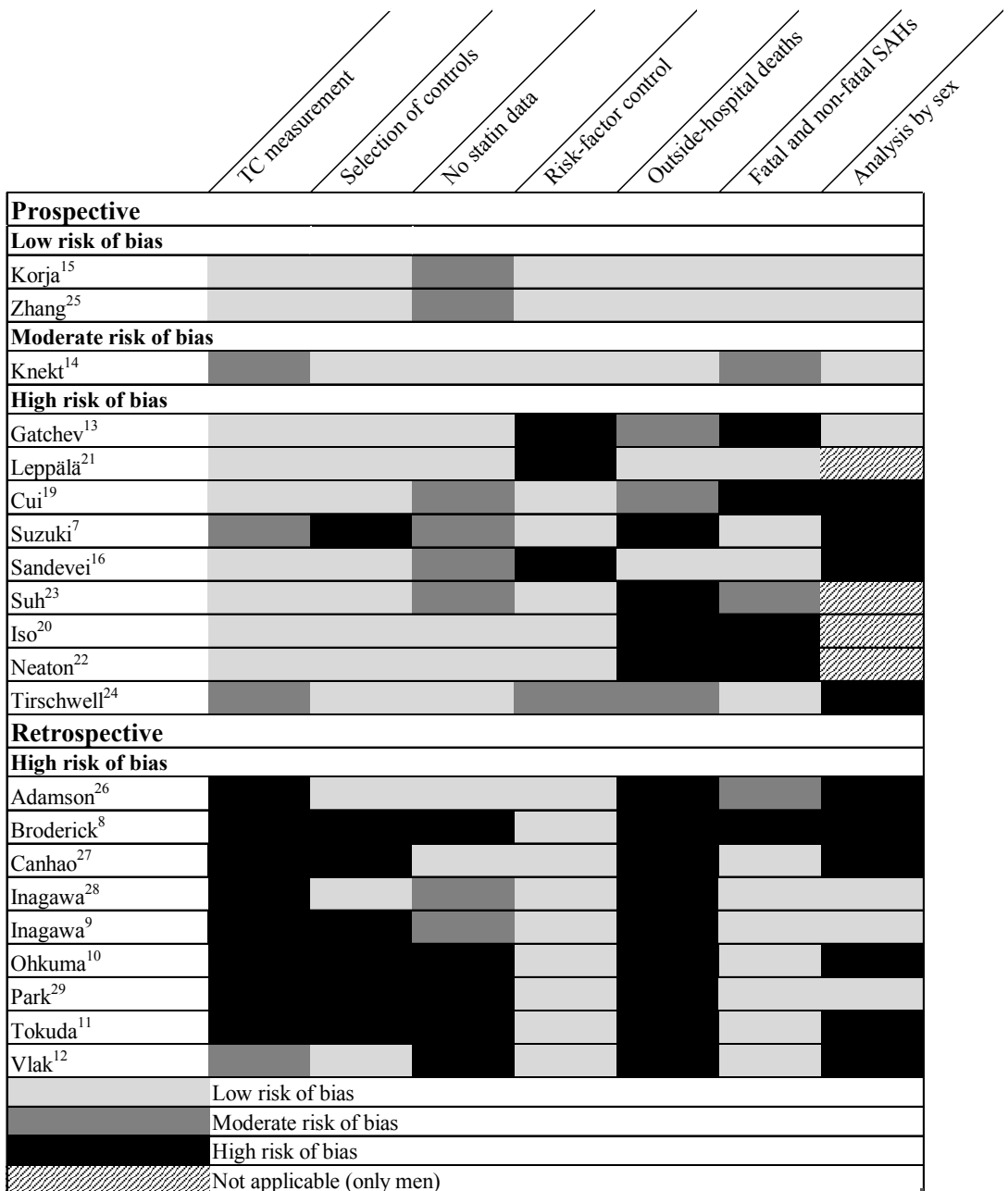
### **6.1.2 Quality assessment**

Only two studies achieved a low risk-of-bias classification, whereas all the others had a high or moderate risk of bias (Figure 2). The two studies originated from Finland, although from different study groups. The oldest study<sup>14</sup> included only very high TC values whereas the most recent study overlapped with the statin era. The main limitations in prospective studies were 1) absence of the 25% of aSAH individuals who die outside hospitals, leading to selection bias, 2) insufficient confounder adjustment, 3) lack of analysis by sex, and 4) no data on lipid-lowering medication (Figure 2). The main limitations among retrospective studies were 1) exclusion of those 25% of aSAH individuals who die outside hospitals, leading to selection bias, 2) cholesterol measurement after aSAH, leading to risk for reverse causality, 3) selection of controls with potentially higher than average TC, 4) imprecise measurement of cholesterol by interview leading to missclassification, 5) lack of analysis by sex, and 6) no data on lipid-lowering medication (Figure 2).

### **6.1.3 Summary of the evidence**

The two low-risk-of-bias studies indicated that high cholesterol may elevate aSAH risk, at least in men. Because of differing study methodologies and considerable limitations in most studies reviewed, Study I was mainly a qualitative review. However, a meta-analysis of the most similar prospective studies supported the findings of low-risk-of-bias studies with RR 1.33 (95%CI 0.94-1.88) for high TC >7.00 mmol/l when compared to low TC <4.90 mmol/l. However, the high-risk-of-bias retrospective studies, which measured cholesterol by interview, suggested that low TC elevates aSAH risk with RR 0.42 (95%CI 0.19-0.95). One of the low-risk-of-bias studies also reported no association between HDL-C and aSAH. No prospective studies investigated associations between LDL-C or apolipoproteins and aSAH. If the low-risk-of-bias studies are indeed the best estimates of TC and aSAH, the PAFs for hypercholesterolemia could reach as high as 35% in the USA and 32% in Europe.

**Figure 2.** Risk of bias in all the 21 studies selected for review.



Adapted from *PLOS ONE*. 2016;11(4):e0152568, Lindbohm et al., “Cholesterol as a risk factor for subarachnoid hemorrhage: A systematic review,” by permission of the Public Library of Science.

## 6.2 FINRISK studies (Studies II-IV)

### 6.2.1 FINRISK cohort characteristics

At enrolment, mean ages were 45.3 (SD 12.1) years for all participants and 45.6 (SD 11.5) years for those who suffered aSAH. For aSAH cases, median follow-up time reached 14.8 years, and for the whole cohort 21.1 years. At enrolment, 38% of men and 17% of women smoked, and pack-years for current smokers at enrolment was 19.0 in men and 10.7 in women (Table 1). Female sudden-death aSAH individuals were older than those women with aSAH who reached hospital. Men who died suddenly from aSAH more often lived alone than did men hospitalized for aSAH (Table 1).

**Table 1.** Differences in baseline risk factors between sudden-death aSAH and hospitalized aSAH individuals. The table describes mean and standard deviation or number and percentages.

	<b>Total cohort</b>	<b>Hospitalized aSAHs</b>	<b>Sudden-death aSAHs</b>	<b>p for difference</b>
<b>Men</b>	31 716	205	46	
<b>Age at enrolment</b>	45.1 (12.3)	45.8 (11.3)	46.4 (10.1)	0.62
<b>Age at end of follow-up</b>	67.3 (12.4)	59.9 (12.7)	59.3 (9.1)	0.76
<b>Alcohol (g/week)</b>	82 (123)	94 (131)	133 (177)	0.36
<b>BMI (kg/m<sup>2</sup>)</b>	26.5 (3.8)	26.1 (3.4)	26.2 (3.5)	0.81
<b>Cholesterol (mmol/l)</b>	6.0 (1.3)	6.3 (1.4)	6.7 (1.3)	0.10
<b>SBP (mmHg)</b>	143 (20)	146 (20)	149 (21)	0.40
<b>Smoking*</b>				
Never-smokers (%)	10 879 (35)	49 (24)	10 (23)	0.56
Ex-smokers (%)	8 228 (27)	52 (26)	8 (19)	-
Smokers (%)	11 910 (38)	100 (50)	25 (58)	-
CPD	17.3 (9.6)	18.5 (8.7)	21.3 (10.7)	0.44
Pack-years	19.0 (16.0)	21.0 (15.8)	29.0 (21.9)	0.33
<b>Marital status*</b>				
Married or cohabiting (%)	24 317 (77)	161 (79)	29 (63)	0.03
No partner (%)	7 324 (23)	44 (21)	17 (37)	-

<b>Stroke in either parent*</b>				
No	26 679 (89)	166 (83)	38 (84)	0.82
Yes	3 248 (11)	34 (17)	7 (16)	-
<b>Education*</b>				
Low	8 269 (27)	72 (34)	14 (31)	0.06
Intermediate	10 915 (35)	66 (32)	22 (49)	-
High	11 826 (38)	72 (34)	9 (20)	-
<b>Women</b>	33 805	240	52	
<b>Age at enrolment</b>	44.9 (12.2)	45.7 (12.1)	47.9 (10.9)	0.25
<b>Age at end of follow-up</b>	69.1 (13.5)	61.9 (12.7)	66.9 (14.1)	0.01
<b>Alcohol (g/week)</b>	27 (50)	38 (82)	40 (60)	0.92
<b>BMI (kg/m<sup>2</sup>)</b>	26.2 (4.9)	26.1 (4.8)	25.9 (4.8)	0.76
<b>Cholesterol (mmol/l)</b>	5.9 (1.3)	6.3 (1.4)	6.3 (1.4)	0.97
<b>SBP (mmHg)</b>	139 (23)	146 (27)	151 (22)	0.35
<b>Smoking*</b>				
Never-smokers (%)	24 084 (72)	148 (63)	28 (56)	0.83
Ex-smokers (%)	3 623 (11)	16 (7)	4 (8)	-
Smokers (%)	5 681 (17)	72 (31)	18 (36)	-
CPD	12.0 (7.1)	13.4 (6.7)	13.8 (7.0)	0.95
Pack-years	10.7 (10.8)	11.9 (10.5)	10.0 (5.4)	0.85
<b>Marital status*</b>				
Married or cohabiting (%)	24 692 (73)	166 (70)	30 (58)	0.09
No partner (%)	9 040 (27)	72 (30)	22 (42)	-
<b>Stroke in either parent*</b>				
No stroke in either parent	28 051 (88)	195 (85)	42 (88)	0.63
Stroke in either parent	3 848 (12)	35 (15)	6 (12)	-
<b>Education*</b>				
Low	10 034 (30)	78 (33)	14 (28)	0.44
Intermediate	11 181 (34)	90 (38)	16 (33)	-
High	11 849 (36)	70 (29)	19 (39)	-

\*For categorical variables a single p-value for within group differences is provided.

Adapted from *Stroke*. 2017 Sep;48(9):2399-2404, Lindbohm et al., “Risk factors of sudden death from subarachnoid hemorrhage,” by permission of the American Heart Association, Inc.



6.2.2 Lipid profile and aSAH risk

Table 2 presents lipoprotein profiles in men and women before and after imputation. Men had a more adverse lipid profile than did women, and LDL-C and ApoB values were on average more adverse after imputations in both sexes. This was expected because we imputed lipoprotein values for individuals who were from an era when average TC in Finland was higher. aSAH patients had more missing lipid values than did other participants because a larger proportion of them originated in an era when lipoprotein measurement methods were unavailable (Figure 3 A and B).

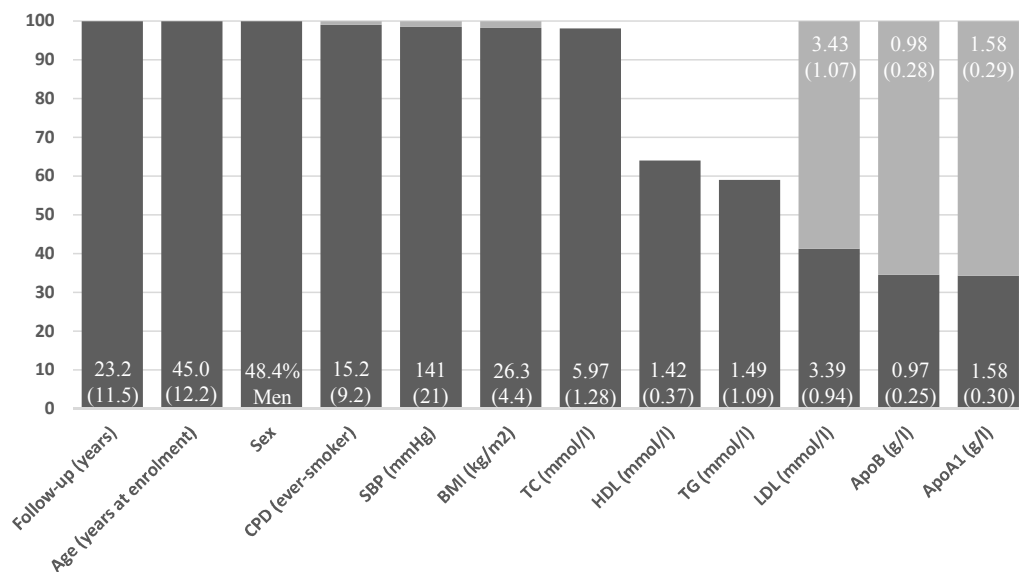
**Table 2.** Lipid-profile characteristics for all individuals at baseline and for incident aSAH cases. (Means and standard deviations)

	Missing	Cohort	aSAHs	Men	aSAHs in men	Women	aSAHs in women
TC							
(mmol/l)	1.9 %	5.97 (1.28)	6.32 (1.39)	6.03 (1.26)	6.39 (1.38)	5.92 (1.30)	6.26 (1.40)
HDL-C							
(mmol/l)	36.1 %	1.42 (0.37)	1.36 (0.39)	1.28 (0.33)	1.24 (0.38)	1.55 (0.37)	1.47 (0.37)
LDL-C							
(mmol/l)	58.8 %	3.39 (0.94)	3.59 (1.02)	3.49 (0.95)	3.75 (1.10)	3.30 (0.92)	3.39 (0.89)
LDL-C							
(mmol/l)							
imputation	0 %	3.43 (1.07)	3.59 (1.19)	3.54 (1.06)	3.73 (1.18)	3.34 (1.08)	3.46 (1.18)
Triglycerides							
(mmol/l)	41.1 %	1.49 (1.09)	1.59 (1.23)	1.73 (1.30)	1.86 (1.56)	1.28 (0.79)	1.34 (0.72)
ApoA1							
(g/l)	65.8 %	1.58 (0.30)	1.55 (0.33)	1.48 (0.26)	1.49 (0.33)	1.67 (0.30)	1.63 (0.33)
ApoA1							
(g/l)							
imputation	0 %	1.58 (0.29)	1.56 (0.31)	1.48 (0.26)	1.47 (0.30)	1.67 (0.28)	1.65 (0.29)
ApoB							
(g/l)	65.5 %	0.97 (0.25)	1.02 (0.27)	1.02 (0.25)	1.07 (0.30)	0.93 (0.24)	0.96 (0.21)
ApoB							
(g/l)							
imputation	0 %	0.98 (0.28)	1.03 (0.29)	1.03 (0.27)	1.10 (0.28)	0.94 (0.28)	0.98 (0.29)

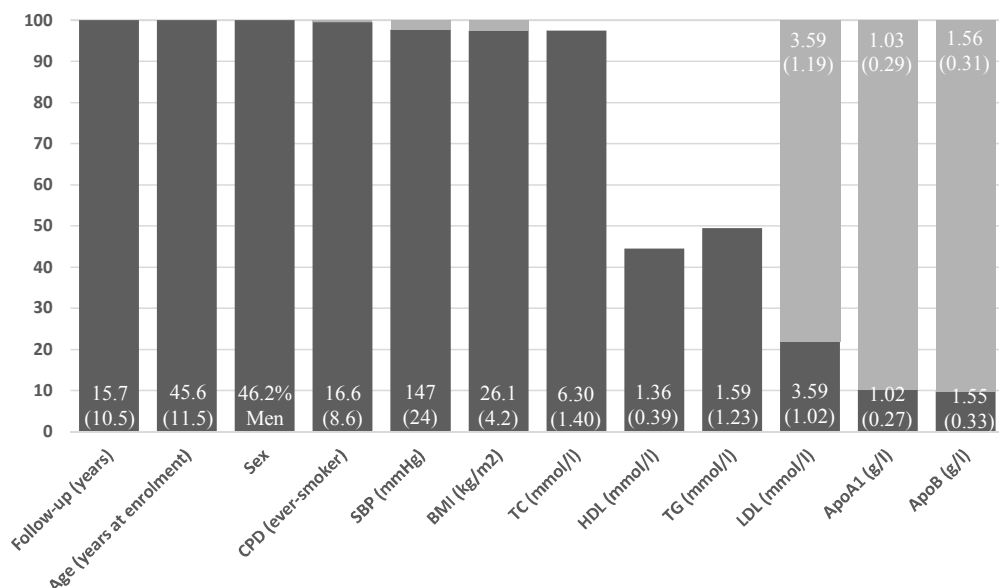
Adapted from *Atherosclerosis*, 2018 Jul;274:112-119, Lindbohm et al., “Adverse lipid profile elevates risk for subarachnoid hemorrhage: A prospective population-based cohort study,” by permission of Elsevier B.V.

**Figures 3A and B.** Percentage of imputed (light grey) and measured (dark grey) values among the whole cohort and among aSAH cases. Numbers in light grey columns: mean and standard deviation values after imputation; dark grey: before imputation. HDL-C and total cholesterol analyses included no imputation, and triglyceride imputation did not converge.

### A) Cohort



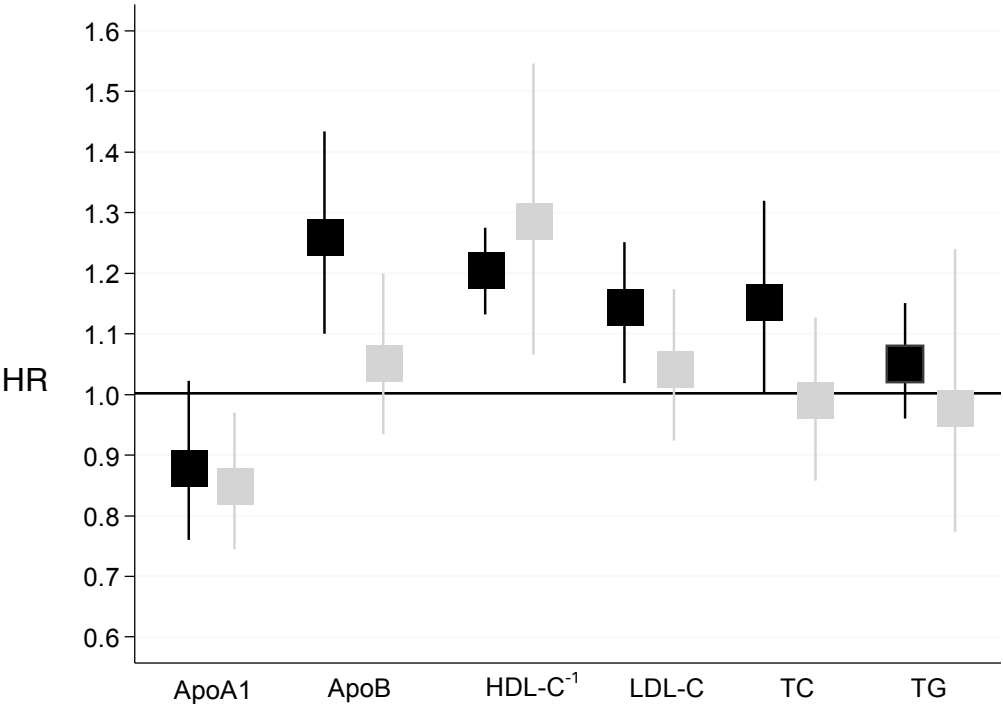
### B) aSAH



Adapted from *Atherosclerosis*, 2018 Jul;274:112-119, Lindbohm et al., “Adverse lipid profile elevates risk for subarachnoid hemorrhage: A prospective population-based cohort study,” by permission of Elsevier B.V.

In our analyses, every SD increase in TC elevated aSAH risk only in men (HR 1.15, 95% CI 1.00-1.32). Every SD decrease in HDL-C elevated aSAH risk in the model, including both sexes (HR 1.20, 95% CI 1.14-1.26), and this association persisted in both men (HR 1.20, 95% CI 1.14-1.27) and women (HR 1.29, 95% CI 1.07-1.55) (Figure 4). The number of aSAH cases with measured LDL-C was too limited for reliable analysis. After imputations, in line with TC results, LDL-C elevated the risk only in men (HR 1.14, 95% CI 1.04-1.25) (Figure 4). Competing risk analyses with complete case data supported these finding by showing that high TC elevates aSAH risk in men, and that low HDL-C elevates the risk in women. PAF analyses further supported our review’s finding by showing that in men, TC > 5 mmol/l

**Figure 4.** Hazard ratio and 95% confidence intervals (whiskers) per standard deviation increase for each lipid variable from multivariate models (adjusted for age, sex, SBP, BMI, smoking, study year, and area) including imputations for ApoA1, ApoB, and LDL-C. Black indicates men and grey women, while y-axis presents hazard ratio scale. Units for ApoA1 and ApoB are g/l and for HDL-C, LDL-C, TC and TG mmol/l.



Adapted from *Atherosclerosis*, 2018 Jul;274:112-119, Lindbohm et al., “Adverse lipid profile elevates risk for subarachnoid hemorrhage: A prospective population-based cohort study,” by permission of Elsevier B.V.

has a PAF of (33%, 95% CI 0-57%) which is comparable to PAFs of smoking (34%, 95% CI 23-44%) and hypertension (34%, 95% CI 0-57%). In women, the corresponding PAFs for smoking, hypertension, and TC > 5 mmol/l were 22% (95% CI 19-25%), 49% (95% CI 30-64%), and 12% (95% CI -16-33%), indicating that smoking and hypertension but not TC play a major role in development of aSAH in women at population level.

Complete-case analysis included too few participants with measurements of ApoA1 and ApoB values. After imputation, each SD increase in ApoA1 reduced aSAH risk in the model combining men and women (HR 0.86, 95% CI 0.78-0.95). This association remained essentially the same in separate analysis of men (HR 0.88, 95% CI 0.76-1.02) and women (HR 0.85, 95% CI 0.74-0.97) (Figure 4). In line with TC and LDL-C results, analysis of ApoB with imputed data showed that every SD increase elevated aSAH risk only in men (HR 1.26, 95% CI 1.10-1.44). Despite these differences, we were unable to show strong evidence of effect modification between any lipid variable and sex. All our HRs remained essentially the same in analysis including only the pre-statin era.

### **6.2.3 Smoking habits and aSAH risk**

Moderate evidence supported elevated aSAH risk in recent quitters (HR 1.93, 95% CI 0.98-3.79) and in former smokers (HR 1.34, 95% CI 0.98-1.82) compared to never-smokers. Female current smokers had a higher aSAH risk (HR 3.43, 95% CI 2.58-4.55) than did men (HR 2.20, 95% CI 1.56-3.10). Among heavy smokers, the HR in women was 8.35 (95% CI 3.86-18.06) compared to HR 2.76 (95% CI 1.68-4.52) in men (Table 3). This indicated multiplicative effect modification ( $p=0.01$ ) between smoking and sex as well as a linear dose-dependent association in both sexes ( $p=0.51$  and  $p=0.30$  for linearity departure in men and women). The association between baseline pack-years and aSAH was also linear in both sexes and stronger in women, in line with CPD results.

Competing risks analysis supported these finding by showing higher life-time aSAH risk in female smokers than in male smokers (Figure 5). When our analyses controlled for the effect modification between smoking and sex, female sex no longer elevated aSAH risk (HR 1.18, 95% CI 0.86-1.62). Additionally, when compared to male never-smokers, female never-smokers did not have an elevated aSAH risk, (HR 1.19, 95% CI 0.85-1.67)

**Table 3.** Risk for aSAH in the smoking categories in men and women. Model adjusted for age, SBP, BMI, total cholesterol, study year, and area.

	Overall HR (95% CI)	No. of aSAHs	Men HR (95%CI)	No. of aSAHs	Women HR (95% CI)	No. of aSAHs
Never-smokers (reference category)	1		1		1	
Former smokers (quit over 6 months earlier)	1.34 (0.98-1.82)	60	1.26 (0.84-1.88)	47	1.14 (0.64-2.04)	13
Recent quitters (less than 6 months earlier)	1.93 (0.98-3.79)	10	1.47 (0.59-3.67)	6	2.57 (0.94-6.97)	4
1-10 CPD	2.54 (1.90-3.40)	63	1.93 (1.17-3.18)	22	2.95 (2.07-4.22)	41
11-20 CPD	2.82 (2.14-3.70)	96	2.13 (1.46-3.11)	61	3.89 (2.63-5.74)	35
21-30 CPD	3.79 (2.51-5.71)	30	2.76 (1.68-4.52)	23	8.35 (3.86-18.06)	7
31 or more CPD	3.91 (1.97-7.75)	9	3.64 (1.79-7.40)	9	-	0

Adapted from *Stroke*. 2016;47(8):1975-1981, Lindbohm et al., “Sex, smoking, and risk for subarachnoid haemorrhage,” by permission of the American Heart Association, Inc.

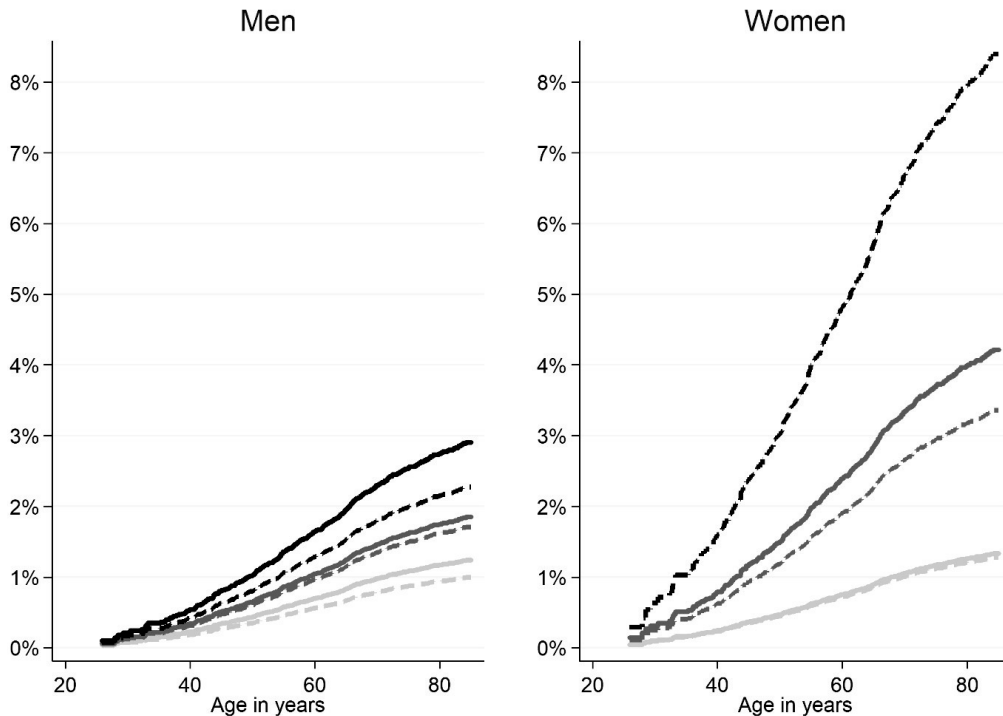
further supporting the evidence that elevated aSAH risk in women is explained by vulnerability to smoking. The effect modification was also present in the additive scale with relative excess risks due to interaction of 2.11 and 6.90 in women smoking 11-20 CPD and >21 CPD.

**6.2.4 Sudden-death aSAHs vs hospitalized aSAHs**

Based on our review, all retrospective and most prospective aSAH studies exclude the approximately 25% of aSAH individuals who die suddenly outside hospitals. If these patients have risk-factor profiles differing from those of hospitalized patients, the hospital-based studies may be unable to provide reliable risk-factor estimates and may fail to detect all aSAH risk factors due to selection bias. We thus studied the differences in risk factors between the 98 sudden-death aSAHs and 445 hospitalized aSAHs included in our dataset.

In relation to BMI, TC, and sex, no strong evidence of differences emerged between hospitalized aSAH and sudden-death aSAH. After controlling

**Figure 5.** Cumulative aSAH incidence in men and women by smoking status. Solid black lines: >30 CPD, dashed black: 21-30 CPD, solid dark grey: 11-20 CPD, dashed dark grey: 1-10 CPD, solid light grey: former smokers, dashed light grey: never-smokers. Model adjusted for age, SBP, BMI, total cholesterol, study year, and area.



Adapted from *Stroke*. 2016;47(8):1975-1981, Lindbohm et al., “Sex, smoking, and risk for subarachnoid haemorrhage,” by permission of the American Heart Association, Inc.

for the interaction between sex and smoking, female sex did not elevate aSAH risk in any analysis model. Interestingly, those who lived without a partner were at elevated risk only for sudden-death aSAH (HR 2.09, 95% CI 1.33-3.28) (Table 4). Moderate evidence suggested that smoking elevates sudden-death aSAH risk more than it elevates hospitalized aSAH risk ( $p=0.05$ ). Sudden-death aSAH risk increased with increasing smoking rates and had HR 5.04 (95% CI 2.22-11.44) in the >20 CPD group, whereas HR for hospitalized aSAH in the same group was 2.93 (95% CI 1.92-4.47). Moreover, moderate evidence supported the finding that high SBP elevated sudden-death aSAH risk more than it elevated hospitalized-aSAH risk ( $p=0.05$ ). The HR for every SD elevation in SBP was 1.34 (95% CI 1.09-1.65) for sudden-death aSAH

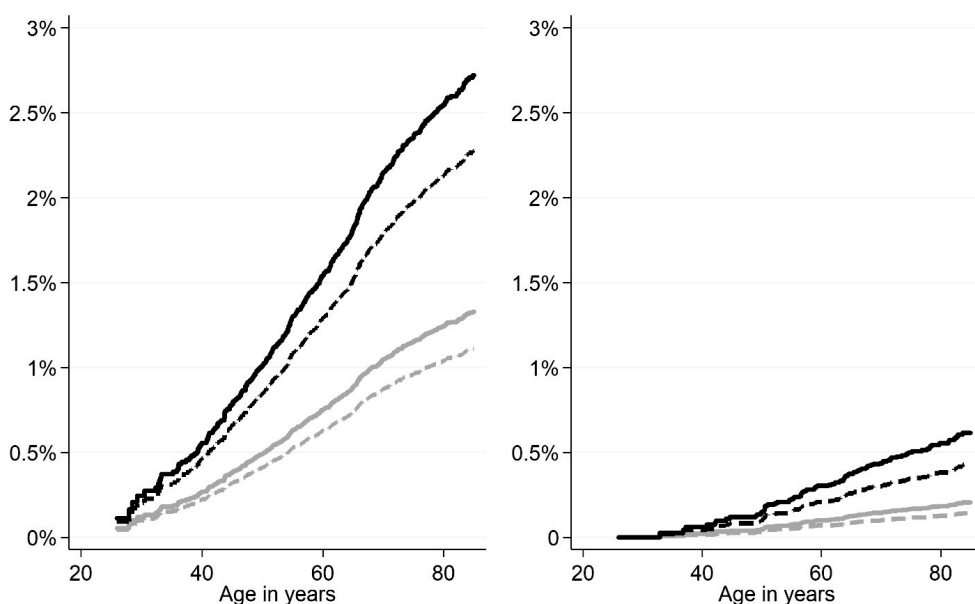
**Table 4.** Risk factor differences between sudden-death and hospitalized aSAHs. Model is adjusted for variables presented (either marital status or living status).

	All aSAHs	Sudden-death HR (95% CI)	Cases (n)	Hospitalized HR (95% CI)	Cases (n)	p for difference
<b>BMI</b>						
(per SD of 4.4 kg/m <sup>2</sup> )	0.90 (0.81-0.99)	0.86 (0.68-1.09)	95	0.91 (0.81-1.01)	434	0.70
<b>Cholesterol</b>						
(per SD 1.3mmol/l)	1.04 (0.95-1.15)	1.10 (0.88-1.37)	95	1.04 (0.94-1.16)	434	0.25
<b>SPB</b>						
(per SD 21.4 mmHg)	1.27 (1.15-1.39)	1.34 (1.09-1.65)	95	1.25 (1.12-1.38)	435	0.05
<b>Sex</b>						
Male	1	1	46	1	205	
Female	1.31 (1.07-1.61)	1.45 (0.90-2.35)	52	1.31 (1.05-1.63)	240	0.68
<b>Smoking</b>						
Never	1	1	38	1	197	
Ex-smokers	1.37 (1.04-1.83)	1.35 (0.65-2.80)	12	1.37 (1.01-1.86)	68	0.91
1-20 CPD	2.50 (2.00-3.12)	3.41 (2.01-5.79)	34	2.34 (1.84-2.98)	142	0.18
Over 20 CPD	3.31 (2.28-4.81)	5.04 (2.22-11.44)	9	2.93 (1.92-4.47)	29	0.10
per 5 cigarettes	1.21 (1.16-1.26)	1.28 (1.17-1.39)	81	1.19 (1.13-1.24)	367	0.05
<b>Marital status</b>						
Married or cohabiting	1	1	59	1	327	
Single	1.09 (0.84-1.42)	1.85 (1.07-3.19)	18	0.99 (0.74-1.33)	58	0.04
Divorced	1.56 (1.13-2.17)	2.83 (1.48-5.41)	12	1.33 (0.92-1.93)	32	0.05
Widowed	1.34 (0.91-1.97)	2.15 (1.02-4.56)	9	1.24 (0.81-1.90)	26	0.17
<b>Living status</b>						
Married or cohabiting	1	1	59	1	327	
No partner	1.25 (1.03-1.53)	2.09 (1.33-3.28)	39	1.01 (0.79-1.28)	116	0.006

Adapted from Stroke. 2017 Sep;48(9):2399-2404, Lindbohm et al., “Risk factors of sudden death from subarachnoid hemorrhage,” by permission of the American Heart Association, Inc.

and 1.25 (95% CI 1.12-1.38) for hospitalized aSAH (Table 4). Cumulative incidence analysis supported these results and indicated that hypertensive smokers were at highest risk for both aSAH types, and that risk was lowest among normotensive never-smokers (Figure 6). Of all aSAHs included, 18% died suddenly outside hospitals. When compared to the reported 25% sudden-death aSAH proportion in Finland, this was somewhat lower, indicating that our cohort may have been somewhat healthier than the general population.

**Figure 6.** Cumulative incidence of hospitalized aSAH (left) and sudden-death aSAH (right). Black line: smokers with systolic blood pressure (SBP)  $\geq 140$  mmHg. Dashed black: smokers with SBP  $< 140$  mmHg, solid grey: never-smokers with SBP  $\geq 140$  mmHg, dashed grey: never-smokers with SBP  $< 140$  mmHg.



Adapted from *Stroke*. 2017 Sep;48(9):2399-2404, Lindbohm et al., "Risk factors of sudden death from subarachnoid hemorrhage," by permission of the American Heart Association, Inc.



## 7 Discussion

Studies I, II, and III suggest resolutions of long-lasting debates on the association between lipid profile and aSAH as well as the association between female sex and aSAH. Additionally, to the best of the authors' knowledge, Study IV is the first to describe risk factors for sudden-death aSAHs in a population-based study. We found that methodological limitations are likely to explain why studies describe low TC as an aSAH risk factor. After minimizing these methodological limitations within the limits of our dataset, an adverse lipid profile associated with elevated aSAH risk. Moreover, limited power and gaps in data analysis are likely reasons for the frequently observed association between female sex and aSAH, which was explained by effect modification between smoking and sex. Furthermore, Study IV on sudden-death aSAH risk factors questions hospital-based studies' ability to identify all aSAH risk factors and also suggests that hospital-based studies may underestimate risk-factor effect sizes.

### 7.1 Lipid profile and aSAH

In Study I, the highest-quality evidence<sup>15,25</sup> indicated that high TC elevates aSAH risk with some evidence of dose dependency, whereas the role played by HDL-C remained unclear. In addition, no study focused on LDL-C and only one retrospective study reported any association between ApoB and aSAH. One of the main findings was the highly variable internal quality of these studies.<sup>7-16,19-29</sup> Only two studies<sup>15,25</sup> received low-risk-of-bias status; these studies did not include reliable statin data, but most of their follow-up occurred in the pre-statin era, which partially excludes the potential effects of statins. In light of the suggested Hills criteria of causality,<sup>131</sup> these two studies<sup>15,25</sup> had the most convincing temporality, biological gradient, plausibility, coherence, and analogy. The sensitivity analyses of studies<sup>15,19,22,24</sup> which included the lowest number of limitations and somewhat comparable TC levels supported the two low-risk-of-bias studies. Other studies had major limitations in 1) exclusion of the 25% of aSAH individuals who die outside hospitals, leading to selection bias, 2) cholesterol measurement after aSAH, leading to risk for reverse causality, 3) selection of controls with potentially higher than average TC, leading to differences between compared groups at baseline, 4)

imprecise measurement of cholesterol by interview, leading to misclassification bias, 5) lack of analysis by sex, or 6) no data on lipid-lowering medication, leading to confounding by indication. If these two studies reliably describe the association between aSAH and TC, the worrying conclusion would be that based on detected prevalence of hypercholesterolemia,<sup>132</sup> TC accounts for 17% to 35% of all aSAHs worldwide. In the high-TC regions such as Europe and the USA, the PAF would be 35% and 32%.

On the basis of findings in Study I, Study II focused on gaps in the literature revealed by Study I. Its results support the evidence provided by Study I and describe associations between lipid profile and aSAH in more detail. All measures of adverse lipid profile elevated aSAH risk in men, whereas in women, only low HDL-C and low ApoA1 elevated the risk. In our analyses, inverse of HDL-C had a stronger association with aSAH than did untransformed HDL-C. This suggests that in addition to other limitations, the studies included in Study I were unable to show any association between HDL-C and aSAH at least partly because they did not include testing for non-linear associations. In our analyses, proportionality remained unviolated, and the results remained the same in sensitivity analysis that included only the pre-statin-era. The PAF analysis was also consistent with that in Study I showing that high TC, high SBP, and smoking each account for one-third of aSAHs, at least in men.

These TC results seem reasonable, because high TC elevates cardiovascular diseases in general,<sup>133</sup> and because atherosclerotic changes appear in aneurysm walls.<sup>44,89</sup> By promoting inflammation in aneurysm walls' smooth muscle cells<sup>88</sup> - the important regulators of the balance between processes of repair and degradation – an adverse lipid profile can cause cell death and make the aneurysms prone to rupture.<sup>44,75</sup>

The TC results observed in Studies I and II in men are in line with Systematic Coronary Risk Estimation risk charts described in the 2016 European Guidelines on cardiovascular disease prevention in clinical practice.<sup>134</sup> Additionally, our findings are comparable to those of prospective studies on abdominal aortic aneurysms, myocardial infarction, and stroke,<sup>135-138</sup> providing evidence that despite differing aetiologies, aSAH belongs to the atherosclerotic disease family. Moreover, when a large prospective Apolipoprotein-related Mortality Risk study<sup>138</sup> combined intracranial haemorrhage and aSAH into the category of haemorrhagic stroke, some evidence of a protective effect from high

HDL-C in women emerged. Contrary to the Apolipoprotein-related Mortality Risk study,<sup>138</sup> Study II was able to include smoking data, and to analyse aSAH separately from intracranial haemorrhage, which could explain the resultant stronger protective association of HDL-C in women.

Even though lipid profile had differing HRs in men and women, we were unable to show any strong evidence of effect modification, possibly due to lack of power. However, if the differences indeed exist, they may result from disparities in development of an adverse lipid profile between the sexes; men may be exposed to an adverse lipid profile for a longer time. Premenopausal women are at lower cardiovascular disease risk than are age- matched men,<sup>133</sup> but physiological changes in menopause elevate cardiovascular disease risk,<sup>139</sup> and cause women's TC and LDL-C levels to peak at approximately age 60.<sup>140</sup> Menopause additionally raises TG and reduces HDL-C, which also contributes to women's elevated cardiovascular disease risk.<sup>140</sup> aSAH incidence studies<sup>7,15</sup> and our competing risk models somewhat support this theory, because they describe aSAH incidence in women as exceeding that of men after age 55.<sup>7,15</sup> However, possibly because of the low number of pre-menopausal women with aSAH, we were unable to show any differences in aSAH in relation to menopausal status. In addition, PAF in Study II is based on the same population as the PAF in Study I, which may limit its external validity if lipid profile associates with aSAH differently in other populations.

However, these results have support from a study that was published after Study I and during the Study-II review process, which suggests that statin treatment reduced aSAH risk.<sup>141</sup> Although that study's results were similar to ours, the authors' complete case analysis included only 10% of the study cohort, meaning that their results rely heavily on an imputation model that the authors did not validate. Additionally, those authors did not study non-linear associations between lipid variables, had no data on duration of statin treatment, excluded high-aSAH-risk individuals by surgery or by endovascular coiling, and were unable to control SES that could relate to statin use and aSAH risk. Although those authors' results are in line with ours, these limitations should be clarified in future and studied in detail before recommending initiation of statin treatment for UIA patients.

## 7.2 Smoking and aSAH

Strong evidence of multiplicative and additive effect modification emerged between smoking and sex, suggesting that elevated aSAH risk in women results from vulnerability to smoking. When the analysis excluded all smokers or controlled for effect-modification between smoking and sex, female sex was not an aSAH risk factor: results remained the same in all analysis models and in all age groups, with reasonable power. When we used the same analysis protocol as that of earlier studies which consistently describe elevated aSAH risk in women,<sup>7,15,16,142</sup> female sex returned as an aSAH risk factor. This was related to lack of control for effect modification and to residual confounding in binary smoking and SBP variables. We also showed, in line with earlier findings, a dose-dependent association between smoking and aSAH in both sexes.<sup>79,80,143</sup> Even 1-10 CPD elevated aSAH risk, but risk decreased after smoking cessation in both sexes, as observed in earlier case-control studies.<sup>79,80</sup> These results also persisted in analysis including competing risks.

Studies on MI and stroke support our findings by describing decreased risk after smoking cessation.<sup>144,145</sup> and by describing a dose-dependent association between smoking and both outcomes.<sup>144,146</sup> However, one recent study, which was published after Study III, suggests that smoking cessation does not diminish aSAH risk.<sup>147</sup> That study used a hospital-based cohort, which excluded all treated aneurysm patients – the ones at the highest aSAH risk. Moreover, that study did not include any measure of time in its analyses and did not perform competing risks analysis. Such an approach limited their ability to study risk factor changes over time and to take into account the competing risk by aneurysm treatment. Additionally their multivariate analysis included many variables with potential collinearity, such as coronary artery disease, years smoked, and age. These limitations may have biased their results, as suggested by the opposite HRs of female sex, family history of aneurysms, age at diagnosis, and coronary artery disease<sup>147</sup> when compared to findings of other aSAH studies.<sup>14-16,148</sup> Associations between smoking cessation and aneurysm rupture when an aneurysm is already present thus demand more research in a more rigorous set-up. However, smoking cessation remains highly beneficial when considering patients' overall prognosis.

Our finding of effect modification between smoking and sex is supported by studies on myocardial infarct.<sup>149,150</sup> The deleterious effect of smoking may relate to decreased oestrogen levels. Smoking can directly reduce oestrogen level and even further reduces it by inducing early menopause.<sup>151,152</sup> The decrease may further lead to collagen depletion, inflammation, and the dysfunction of mural cells in vessel walls,<sup>75</sup> which is a major degrading cascade in vessel walls.<sup>44,46</sup> These changes may then contribute to aneurysm formation and aSAH.<sup>46</sup>

### **7.3 Sudden-death aSAHs**

To the best of the authors' knowledge, Study IV described risk factors of sudden-death aSAHs for the first time in a population-based cohort. As expected, high SBP and smoking elevated risk for sudden-death aSAH just as they elevate risk for hospitalized aSAHs. The novel findings were that high SBP and smoking elevate sudden-death aSAH risk more than they elevate hospitalized-aSAH risk, and that those who live alone are at elevated sudden-death aSAH risk. All the associations were similar in both sexes, but the number of sudden-death aSAHs was small. These findings indicate that a more adverse risk-factor profile and living alone associates with higher sudden-death aSAH risk, and that hospital-based studies may not include those at highest aSAH risk. Hospital-based studies may thus somewhat underestimate aSAH risk factors suggesting that current UIA-treatment guidelines<sup>17,18</sup> may underestimate the role played by risk factors. Additionally, the reason that hospital-based studies may not detect all aSAH risk factors may be in part selection bias.

Studies on sudden-death myocardial infarct<sup>153,154</sup> support our findings and describe a more adverse risk-factor profile in sudden-death myocardial infarct than in hospitalized myocardial infarct patients. Our findings are also plausible on a biological basis since a more adverse risk factor profile may hasten the degrading processes in aneurysm walls and lead to more severe bleeds. In addition, those with a more adverse risk factor profile may also have more comorbidities that could lead to a higher mortality rate at the onset of aSAH. The elevated risk among those who live alone may also relate to social isolation and to more adverse development of health and risk factors during follow-up than for those who live with a partner.<sup>155</sup> Another possible explanation for elevated aSAH risk in those who live alone is that, because

of disabling symptoms of aSAH and lack of potential helpers, they are less likely to receive medical attention. This could be a plausible explanation if aSAH behaved like sudden-cardiac deaths of which 80% occur at home, with of those only 60% being witnessed.<sup>154</sup> Thus, individuals carrying a UIA and living alone may benefit from more aggressive risk-factor reduction efforts and social support and possibly also from different treatment approaches from those approaches for those who live with a partner or spouse. Finally, even though our cohort had a good participation rate, we cannot exclude the possibility that selection bias led to underestimation of associations between sudden-death aSAH and risk factors if those with the most severe risk factors and a potentially higher aSAH rate did not participate.

## 7.4 Strengths

Study I had an extensive search strategy which provided 21 studies with over 50 aSAHs. When compared to earlier systematic reviews identifying only seven,<sup>87</sup> six,<sup>77</sup> and three<sup>156</sup> studies with a total of over 50 aSAH cases, Study I is the most extensive to date. Moreover, contrary to previous systematic reviews, Study I focused specifically on qualitative methodological differences instead of being a quantitative summary as recommended by the Cochrane Collaboration when considerable methodological differences are present in the studies selected.<sup>109</sup>

In Studies II to IV using the FINRISK cohorts, a comprehensive measurement of lipid profile allowed more extensive lipid profile analyses than was possible in earlier studies. FINRISK cohorts collected from different decades also allowed us to take into account potential confounding of statin medication by separate analysis of pre- and post-statin eras. Additionally, when compared to earlier studies, our Studies II, III, and IV had robustness in long follow-up, were of sufficient sample size, and had a population-based design reducing the risk for selection bias. All our data were collected at baseline, reducing risk for information bias and reverse causality. Additionally, the FINRISK questionnaire on smoking habits was more extensive than those ones used in earlier studies, enabling us to describe the association between smoking and aSAH in detail. Contrary to most earlier studies, smoking status in the FINRISK cohorts has also been validated against cotinine measurements.<sup>116</sup> Additionally, with the detailed death records in Finland we were able to

include out-of-hospital aSAH deaths with a high 80% autopsy rate, and with 100% diagnostic confirmation for sudden-death aSAHs.<sup>157</sup> To the authors' knowledge, Studies II to IV have the largest number yet of confirmed sudden-death aSAHs and thus offer low risk for major selection bias, suggesting that our results may be more reliable than are those acquired from earlier cohort studies.

## 7.5 Limitations

Despite its exhaustive search strategy, Study I may lack some relevant publications due to limitations in the search strategy and to indexing errors. It also includes a study by two of its authors (M.K. and J.K.) which could have introduced an unintentional conflict of interest in risk-of-bias estimates. To compensate for these limitations, the International Prospective Register of Systematic Reviews database includes the predefined protocol of Study I. Study I also lacks reliable meta-analysis because of differences and limitations in studies reviewed and because of lack of individual patient data. In Study II the proportion of missing data was high, and we needed to use multiple imputation to predict a high percentage of LDL-C and apolipoprotein values. Even though the distributions, mean and SD values, and HRs originating from the imputed data were similar to the measured data, and are plausible based on TC values of those whose data was imputed, these results need careful interpretation, because they rely on predicted values. The common limitation in the lipid profile of Studies I and II is that the best evidence in both studies stems from only one nationality; this weakens the external validity of the results considerably. However, the results of Study I and II are biologically plausible, and three supporting, but high-risk of bias studies do exist that were conducted in the USA.<sup>22,24,141</sup>

The data used in Studies II, III, and IV comprised only baseline measurements, thus indicating that we had limited ability to study risk factors' changes over time. Although only a minor violation in the Cox proportionality assumption regarding SBP emerged, birth cohort analysis suggests that during long follow-up roughly half the smokers quit.<sup>78</sup> These findings suggest that our results may underestimate the risk-increasing effects of smoking and SBP.

In addition, the small number of never-smokers with high alcohol consumption inhibited reliable inclusion of alcohol consumption in adjusted

models.<sup>15</sup> This would bias our results on effect modification if female smokers drank an equal or nearly equal amount of alcohol as men do, because women may be more vulnerable to the adverse effects of alcohol in relation to stroke.<sup>158</sup> However, female aSAH individuals in our cohort drank considerably less than men implying that alcohol consumption may not have greatly confounded or modified our results regarding smoking. Moreover, when our analyses included alcohol consumption and/or years of education as a proxy for SES, which relates to alcohol consumption and other cardiovascular disease risk factors<sup>159,160</sup> our effect estimates and effect modification results remained unchanged.

Additionally, our data did not include confirmed information on familial aSAHs, but when the adjusted model included also self-reported stroke of any kind in either parent, the results still were the same. Furthermore, in all studies from II to IV, some traumatic aSAHs, those included due to indexing errors, may have weakened the associations observed. Study IV on sudden-death aSAH had no data on individual treatment delays, which could bias our results if smoking or high SBP associated with these delays. However, our results on sudden-death aSAH endured when our analysis model included education as a proxy for SES and marital status, both of which may relate to faster recognition of aSAH and thus to shorter treatment delays. Because recent studies indicate that aSAH incidence in Finland is similar to that of other countries,<sup>56,66</sup> the results of Studies II, III, and IV are thus likely to be generalizable to populations of European ancestry with similar cardiovascular morbidity.



## 8 Conclusions

1. Methodological limitations in earlier studies explained at least in part the paradox stating that low TC elevates aSAH risk.
2. Adverse lipid profile elevated aSAH risk and was a major risk factor for men at the population level.
3. Vulnerability to smoking explained at least in part the elevated aSAH risk in women. In both sexes smoking dose-dependently elevated aSAH risk, and quitting smoking reduced risk.
4. Adverse risk-factor profile elevated sudden-death aSAH risk more than it elevated hospitalized aSAH risk. Hospital-based studies may not detect all aSAH risk factors and may somewhat underestimate the associations of those observed.

## 9 Recommendations

This thesis suggests that methodologically limited aSAH risk-factor studies on lipid profile and female sex have led to questionable, long-lasting *status quo* that can be hard to change. As neurosurgical meta-analyses,<sup>161</sup> many aSAH risk factor studies would also benefit from more detailed attention to epidemiological and statistical study methodology that includes more thorough consideration of bias, confounding, competing risks, interaction, effect modification, non-linearity, and time-varying covariate analysis. Because accumulating evidence supports the theory that an adverse lipid profile elevates aSAH risk, future studies should aim to replicate our findings in different populations, with rigorous methodology, and should try to clarify whether lipid-lowering medication can reduce aSAH risk in UIA individuals.

This thesis also suggests that vulnerability to smoking explains, at least in part, elevated aSAH risk in women. The prevailing theory relates elevated aSAH risk to reduced oestrogen levels, and future studies should further investigate this separately in both pre- and post-menopausal women. This is important to clarify because of widespread prescription of hormone replacement therapy and oral contraceptives. Optimally, future aSAH studies should aim at the most unbiased set-up and should include detailed data on aSAH risk factors, sudden-death aSAHs, competing risks, major confounders, menopause status, hormonal replacement therapy, and oral contraceptives, and should measure individual oestrogen levels. These studies could further elaborate on whether or not low oestrogen levels, hormonal replacement therapy or oral contraceptive use associates with aSAH risk in women.

In addition to patient and aneurysm characteristics, treatment decisions regarding UIAs should at least include assessment of the following risk factors: age, sex, SBP, detailed lipid profile, and detailed smoking status and history, and should consider also including marital status. Analyses of future risk-factor studies should focus on men and women separately and include analysis of non-linear associations and potential interactions or effect modifications that are biologically plausible. In addition, future studies should focus on the interplay between different aSAH risk factors by using analysis methods that can cope with mediation and effectively utilize repeated measurements of risk factors. Additionally, with competing risk analysis,

future UIA-treatment guideline consortiums should assess how censoring by treatment of high-aSAH-risk individuals has influenced risk-factor estimates in studies of UIA rupture risk. This approach could potentially change the current guidelines<sup>18</sup> by giving more weight to risk factors. The role of risk factors in UIA treatment decisions is likely to grow further, if future studies could include also the most severe forms of aSAH: sudden-death aSAH. Implementation of these measures has the potential to further expand knowledge of the epidemiology and aetiology of aSAH and reduce aSAH morbidity and mortality world-wide.

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